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SUSCEPTIBILITY OF THE HEART OF THE RABBIT TO SPECIFIC INFECTION IN VIRAL DISEASES

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Some time ago it was reported [1] that in rabbits inoculated peripherally with virus III cardiac lesions developed which on histologic examination contained, in the cells of the exudate and in the parenchymal cells, the intranuclear inclusion bodies typical of virus III infection. If it is granted that the observation of these inclusion bodies constitutes proof of the presence of the virus in the lesion and if, as was the case, no comparable cardiac lesions are encountered in rabbits which are not infected with virus III, it is reasonable to assume that the virus was the etiologic agent responsible for the lesion. Since this is the first demonstration of the cardiac localization of a virus introduced into the body at a site anatomically far removed from the heart, it is of interest to know whether the ability to lodge there is a specific quality inherent in this particular virus or whether it might be a property shared with a number of dissimilar and unrelated viruses.

In favor of the latter view is the fact that cardiac lesions in virus III infection occur in by far the greatest number and severity in animals which have previously undergone some procedure directed toward damaging or overburdening the heart. The work to be described in the present paper was undertaken, therefore, to determine primarily whether the ability to localize in the heart is characteristic of virus III only or is common to all viruses which have no established tropism toward an organ or a tissue and to discover secondarily in what way the lesions, if they occur, differ from one another.

METHOD AND MATERIAL

The rabbit was chosen as the experimental animal not only because it was used in the original work with virus III, of which it is the sole known host, but

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1. Pearce, J. M.: Arch. Path. **28**:827, 1939.

because it is easily infected with several other available viruses. All the rabbits were young males weighing between 1,500 and 3,000 Gm., the majority weighing around 2,500 Gm. No attempt was made to select them according to breed or color, and they were obtained from several different sources.

In addition to being easily infected by virus III, rabbits have been demonstrated to be infected readily by vaccine virus, two strains of fibroma virus, i. e., Shope's original strain A [2] and Andrewes' inflammatory strain,[3] pseudorabies virus (Aujesky [4]), virus myxomatosum (Sanarelli [5]) and the papilloma virus (Shope [6]). The only one of these which has been shown to have any strong tropism for tissue is the papilloma virus, which has been demonstrated repeatedly to be capable of infecting only epidermal tissue (Shope [6]; Rous [7]). For this reason it was not used in the present experiments.

Five groups of 15 rabbits each were used. Three of the groups were used for studies with, respectively, myxoma virus, fibroma virus strain A and the inflammatory fibroma virus, rabbits in these groups being inoculated both intratesticularly and subcutaneously. A fourth group was used for a study with vaccine virus, rabbits being inoculated only intratesticularly. A fifth group was used for a study with the pseudorabies virus, rabbits being inoculated only subcutaneously.

The vaccine virus, the myxoma virus and the two strains of fibroma virus were each inoculated in the form of a 5 per cent suspension of infected testis. The suspensions were prepared by grinding the testis in a mortar with sterile sand in a small amount of 0.85 per cent sodium chloride solution. The resulting paste was then diluted to 5 per cent with additional sodium chloride solution. In the case of the pseudorabies virus the inoculum was a 5 per cent suspension of infected brain which had been stored in glycerin for a short time. The pseudorabies virus was administered in 0.5 cc. doses; the others, in amounts between 1 and 5 cc., distributed between several sites of injection. In no instance did there prove to be any relation between the size of the infecting dose and the incidence of the severity of the cardiac lesions.[8]

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2. Shope, R. E.: J. Exper. Med. **56**:793, 1932.

3. Andrewes, C. H.: J. Exper. Med. **63**:157, 1936. Shope, R. E.: ibid. **63**:173, 1936.

4. Aujesky, A.: Centralbl. f. Bakt. (Abt. 1) **65**:256, 1902.

5. Sanarelli, G.: Centralbl. f. Bakt. (Abt. 1) **23**:865, 1898.

6. Shope, R. E.: J. Exper. Med. **58**:607, 1933.

7. Rous, P., and Beard, J. W.: J. Exper. Med. **60**:701, 1934.

8. The myxoma virus, the pseudorabies virus and the two strains of fibroma virus were procured from Dr. Richard Shope, of the Rockefeller Institute. The myxoma virus was the strain originally obtained from Dr. Arthur Moses, of the Oswaldo Cruz Institute, in Brazil, and used by both J. R. Hobbs (Am. J. Hyg. **8**:800, 1928) and T. M. Rivers (J. Exper. Med. **51**:965, 1930) and maintained by Dr. Shope in the laboratories of the Rockefeller Institute in Princeton since 1932. The strain A fibroma virus was that originally isolated by Shope,[2] in 1932. The inflammatory fibroma virus was the variant described by Andrewes [3] in 1936 and sent by him to Shope. The vaccine virus which was used was a highly virulent strain isolated from a spontaneously infected rabbit in 1938 by me (J. Infect. Dis. **66**:130, 1940). The pseudorabies virus was the Hungarian strain which had been sent to Shope by Aujesky from Budapest in 1930.

The myxoma virus and the strain A fibroma virus had been maintained by both intratesticular and subcutaneous serial passage through rabbits and by storage in 50 per cent glycerin. The vaccine virus and the inflammatory fibroma virus had been carried by testicular passage and storage in glycerin, and the pseudorabies virus, by serial intracerebral inoculation and storage in glycerin.

In the experiments with virus III [1] it was evident that although cardiac lesions developed in a small proportion of rabbits inoculated with the virus intranasally, intratesticularly or intravenously, the heart was seldom affected and never seriously damaged unless the animal had been given an intravenous injection of a solution of acacia or pitressin or had had a needle introduced into the myocardium. Of these three methods of increasing the incidence of cardiac localization of the virus, the intravenous injection of 20 to 50 cc. of a sterile solution of acacia immediately preceding the intratesticular inoculation of the infectious agent had proved to be the most successful. For this reason the intravenous injection of a solution of acacia was used as the localizing procedure in the experiments with the viruses used in the present work.

Immediately preceding the viral inoculation 35 to 50 cc. of a 20 per cent solution of sterile acacia in 0.85 per cent sodium chloride solution or in distilled water was injected into a marginal ear vein of each of 10 animals in each of the groups of 15. The remaining 5 in each group served as controls. The acacia

TABLE 1.—*Incidence of Cardiac Involvement During Infection by Several Viruses*

Virus	Infected Rabbits	Number with Significant Lesions of the Heart	Per Cent
Rabbits Prepared by Intravenous Injection of a Solution of Acacia			
Vaccine virus	10	8	80.0
Pseudorabies virus	10	7	70.0
Fibroma virus (inflammatory strain)	10	8	80.0
Myxoma virus	10	6	60.0
Fibroma virus (strain A)	10	7	70.0
Rabbits Which Had Not Received the Solution of Acacia			
Vaccine virus	5	1	20.0
Pseudorabies virus	5	2	40.0
Fibroma virus (inflammatory strain)	5	1	20.0
Myxoma virus	5	3	60.0
Fibroma virus (strain A)	5	0	0

solution was made from the acacia U. S. P. powder. It was sterilized in the autoclave at 13 pounds' (6 Kg.) pressure for twenty minutes. Samples cultured on the usual bacterial mediums failed to show growth after twenty-four or forty-eight hours.

Each animal was examined immediately after being killed or, if it had died in its cage, within at least twelve hours after death. The autopsy always included a histologic examination of the heart, the testes, the subcutaneous site of inoculation and any other organ or tissue which macroscopically appeared altered. The lungs, adrenals, kidneys, liver and spleen were examined on occasion. Tissues were fixed in Zenker's solution and stained routinely with hematoxylin and eosin. In many instances Giemsa's stain or Masson's [9] trichrome stain was used in addition. The heart was examined externally in the gross but not opened since it had been found previously [1] that more satisfactory sections for microscopic study could be obtained by fixing the intact organ and later trimming it in such a way as to include all four chambers and one or more valves in their usual relationship. By cutting several parallel blocks from a single heart it is not difficult to show at least three of the valves and often four without resorting to serial sections.

9. Masson, P.: J. Tech. Methods **12**:75, 1929.

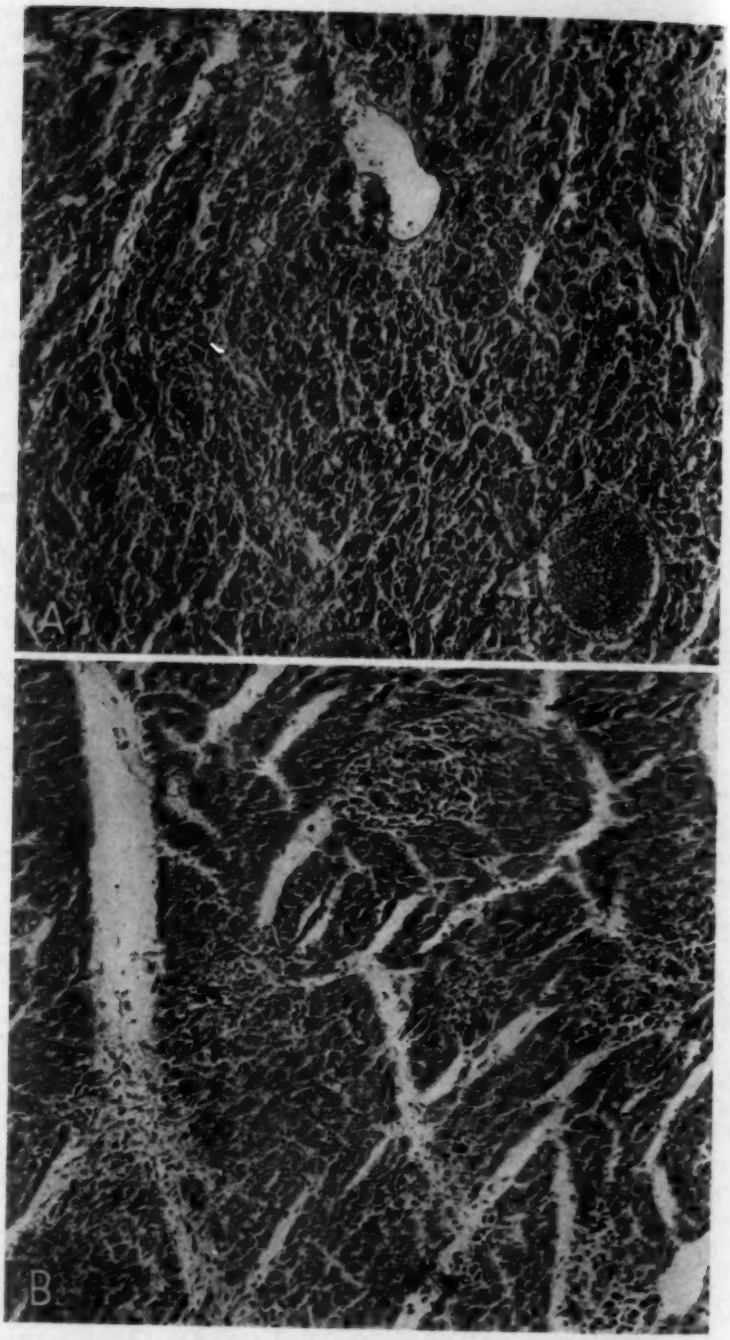

Figure 1

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RESULTS

Vaccine Virus.—Eight of the 10 rabbits given an injection of acacia solution and infected with vaccine virus died with generalized vaccinia three or four days after inoculation. The remaining 2 were killed while moribund on the third or fourth day. As seen in table 1, microscopic examination revealed definite cardiac lesions in 80 per cent. These lesions consisted for the most part in a more or less severe infiltration of the myocardium and the valves by polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, with the latter two types predominating. In the myocardium the infiltration was either diffuse (fig.1 *A*) or in the form of miliary collections of cells around minute foci of myocardial necrosis (fig. 1 *B*). Although lesions occurred in all parts of the myocardium, they were more numerous in the right ventricle and right auricle and in the interventricular septum. Occasionally more extensive areas of necrosis were seen, and in these calcium was deposited. In 2 instances there was some infiltration of the mural endocardium. Lesions were observed in the tricuspid and mitral valves only. Here there occurred an interstitial infiltration of leukocytes together with edema and a proliferation of fibroblasts which often resulted in marked thickening of the flap (fig. 2). Hemorrhages were commonly found in the substance of the valves, but fibrinoid vegetations were not encountered on their surfaces.

Of the 5 infected rabbits which did not receive acacia, 4 died between the fifth and the ninth day after inoculation and 1 was killed on the third day. In 4 there were no significant cardiac lesions. One heart contained small scattered collections of lymphocytes in the ventricular myocardium.

Pseudorabies Virus.—The animals infected with the pseudorabies virus died uniformly on the third day after subcutaneous inoculation. Seven of the 10 which were given acacia showed anatomic evidence of the localization of the virus in the heart. The lesion was predominantly myocarditis but, as seen in table 2, the valves were sometimes inflamed, and in 1 instance extensive hemorrhage, necrosis and polymorphonuclear leukocytic infiltration occurred in the mitral valve. The alteration in the heart muscle was not dissimilar to that seen in vaccinia; somewhat patchy but frequently confluent areas of leukocytic infiltration and disappearance

EXPLANATION OF FIGURE 1

A, diffusely scattered leukocytes and atrophic muscle fibers in the wall of the right ventricle of the heart of rabbit A1, which died three days after being inoculated intratesticularly with vaccine virus. Hematoxylin and eosin; × 128.

B, small foci of muscle necrosis surrounded by mononuclear and polymorphonuclear leukocytes in the interventricular septum of the heart of rabbit 6A, which died four days after being inoculated intratesticularly with vaccine virus. Hematoxylin and eosin; × 128.

Fig. 2.—Leukocytic infiltration, hemorrhage and fibroblastic proliferation in the tricuspid valve of the heart of the same rabbit as in figure 1 *B*. Hematoxylin and eosin; × 142.

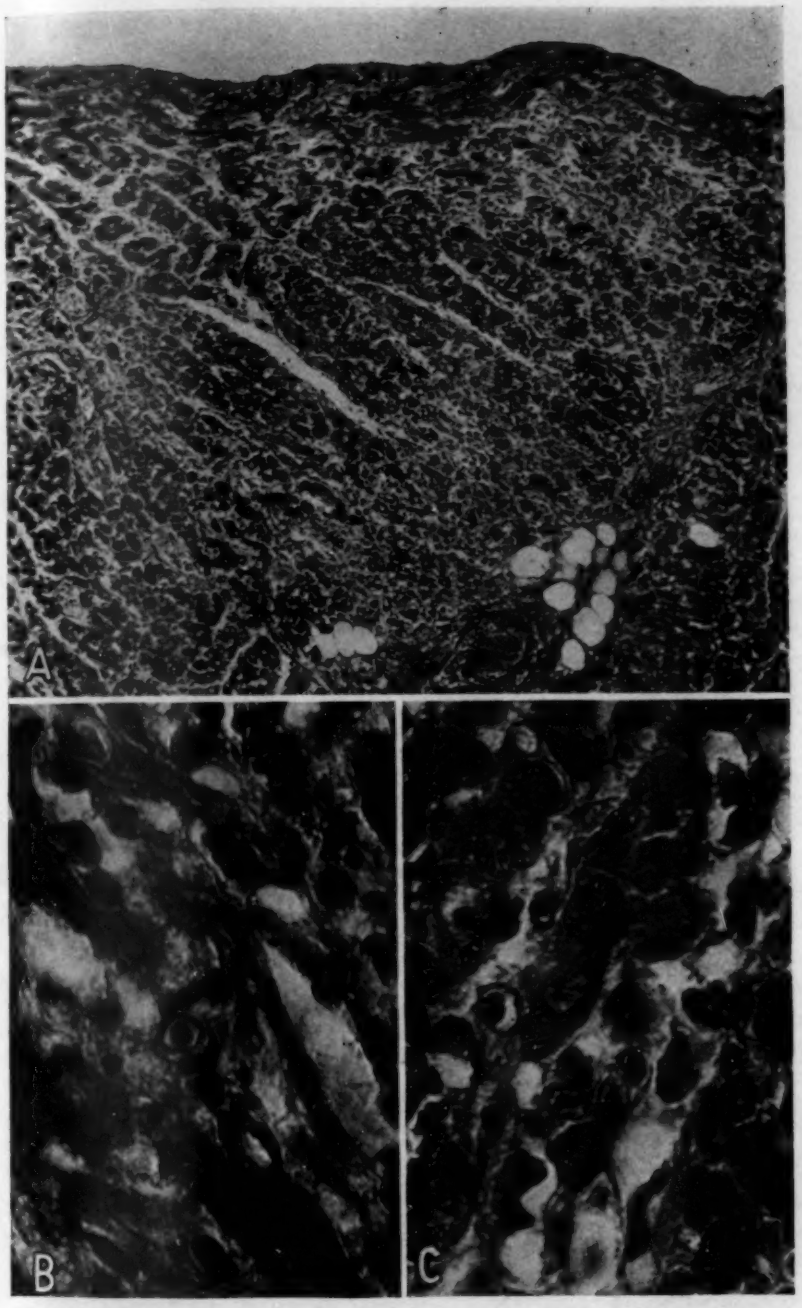

Fig. 3.—*A*, confluent areas of myocardial necrosis and leukocytic infiltration in the wall of the right ventricle of the heart of rabbit B2, which died three days after being inoculated subcutaneously with pseudorabies virus. Hematoxylin and eosin; × 122. *B* and *C*, intranuclear inclusion bodies in the myocardium of rabbit B2. Hematoxylin and eosin; × 871.

of muscle cells were spread through the right side of the heart (fig. 3 *A*). Fibroblasts were scattered sparsely through these areas, and in them and in the mononuclear cells of the exudate the typical intranuclear inclusion bodies of the disease were readily made out. Several inclusions are

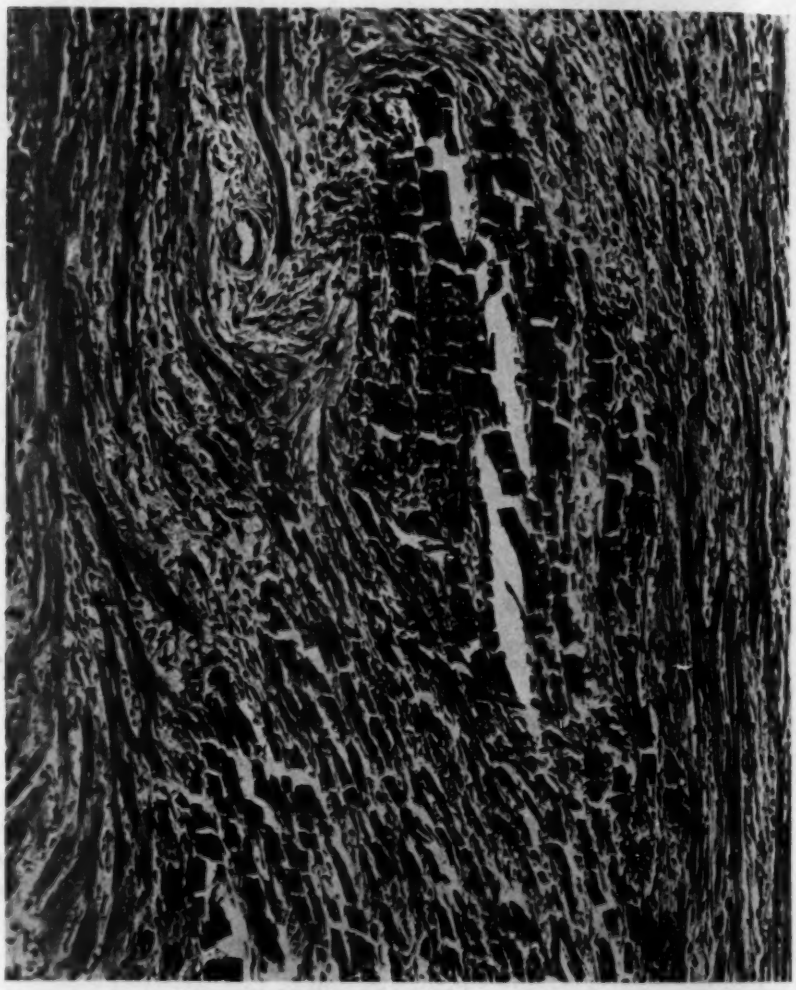

Fig. 4.—Calcification of necrotic muscle fibers near an arteriole in a papillary muscle of the left ventricle of the heart of rabbit C7, which was killed five days after being inoculated intratesticularly with inflammatory fibroma virus. Hematoxylin and eosin; × 122.

shown in figure 3 *B* and *C*. Not uncommonly rows of swollen endothelial cells containing intranuclear inclusion bodies lined parts of the auricles, but again there was no vegetative endocarditis.

In 4 of the rabbits in the control group there were hemorrhages in the tricuspid or the mitral valve, and in 2 of them small foci of mononuclear leukocytes and occasional polymorphonuclear leukocytes lay in the myocardium or the endocardium. No inclusion bodies could be found in any of these lesions.

Inflammatory Fibroma Virus.—This virus killed none of the experimental animals and seldom induced much fever, although the orchitis, as evidenced by enlargement and induration of the testicle during life and by infiltration and necrosis of the parenchyma on histologic examination, was severe. The rabbits were put to death five to eight days after inoculation. Cardiac lesions occurred in 80 per cent of those prepared with acacia, and in many the inflammatory and necrotizing process was severe. Calcification of the necrotic muscle fibers was a common feature (figs. 4 and 5 *A*). Proliferation of large basophilic fibroblasts accompanied the inflammatory reaction in many lesions, and in 3 instances this was especially marked just beneath the epicardium and in the subepicardial fat. Sometimes the infiltrating and proliferative reactions were present in about equal intensity throughout large areas of heart muscle, especially in the auricles (fig. 5 *B*). The necrotizing lesion tended to be focal and to be commoner in the ventricular muscle. The right side of the heart was more frequently the site of lesions than was the left. In 1 rabbit (C 7) (table 2) the mitral valve was extensively involved, and here the change consisted predominantly in the fibromatous reaction, although there were also infiltrating mononuclear cells and small hemorrhages.

One of the 5 animals which did not receive acacia had lesions in the myocardium which, although small and scattered, were in all other respects similar to those in the acacia-prepared group. The remaining 4 had no lesions.

Myxoma Virus.—Of the myxoma-infected rabbits, 10 died between the sixth and the tenth day after inoculation, and 5, obviously moribund, were killed on the seventh and the tenth day, respectively. Six of the acacia group showed well marked involvement of the myocardium by the myxomatous tissue typical of the disease, and in 3 of the 6 the endocardium and the epicardium were also involved, but in each instance this seemed to be a direct extension of the proliferating myxoma cells from the contiguous intramyocardial lesion. The valves were never affected. The type of cellular reaction was identical with that seen in myxoma virus infection in other situations. There were the characteristic large, irregularly stellate or spindle-shaped basophilic cells with huge pale nuclei, in which the chromatin was finely stippled or collected in larger granules at the prominent nuclear membrane (fig. 6 *A*). A variable amount of palely basophilic mucoid material lay between the cells. Mitotic figures were frequently encountered. Scattered throughout the myxomatous areas there were many nuclear fragments and moderate

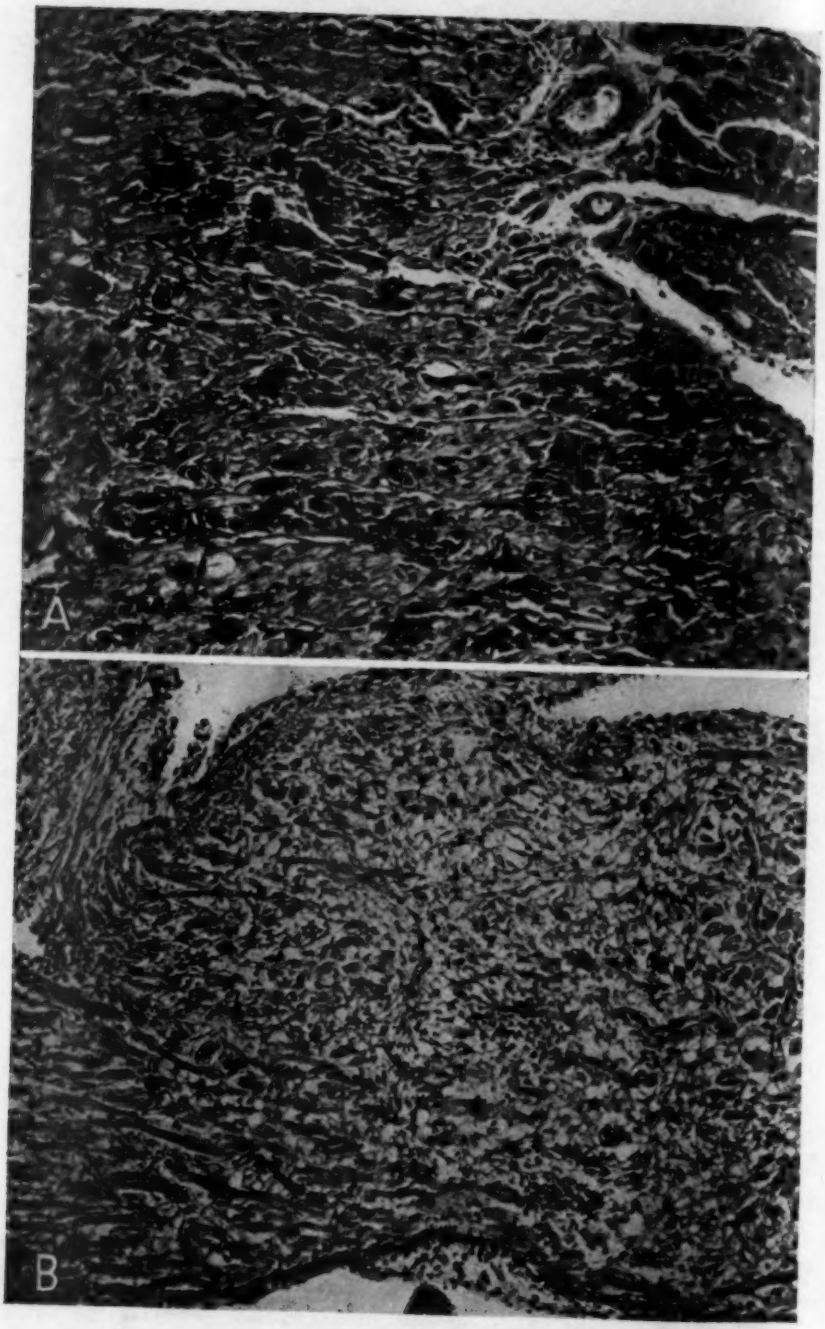

Figure 5

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numbers of polymorphonuclear leukocytes, lymphocytes and mononuclear cells. The heart muscle fibers were frequently atrophic or had disappeared entirely, but in spite of their diminution the thickness of the ventricular wall was increased by the abundant newly formed tissue. The myxoma cells tended to infiltrate everywhere between the muscle fibers and to fill the perivascular spaces and endocardial crevices. The right side of the heart was more severely affected than the left. Figure 6*B* illustrates the involvement of the endocardium and the spread of myxoma cells across an endocardial crevice. The scattered polymorphonuclear leukocytes and indeterminate nuclear fragments are clearly visible here and in figure 6*A*.

Three of the animals which were not treated with acacia had small and infrequent but unmistakable myxomatous lesions in the myocardium of the ventricles and just beneath the endocardium. It is apparent that although the incidence of cardiac involvement is the same in the two groups, the severity of the lesions is much greater in those rabbits prepared by intravenous injection of a solution of acacia.

Fibroma Virus, Strain A.—Shope's original strain of fibroma virus was the least effective of all those studied in producing cardiac lesions. Although a definite histologically demonstrable fibroma virus reaction developed in the hearts of 7 of the 10 acacia-prepared animals (table 2), in only 2 was it at all extensive. In these the lesion was a diffuse proliferation of connective tissue between and around the muscle fibers of the right ventricle and in the areolar tissue surrounding the smaller branches of the coronary arteries (fig. 7). There was no necrosis of muscle, and only occasionally was there an inconspicuous and often focal infiltration of lymphocytes. In the milder lesions the newly formed fibroblastic tissue was in the perivascular areas or in small patches throughout the myocardium. The valves were never affected, and in only 2 instances did the lesions spread to the endocardium. In all 15 animals the typical fibromatous reaction occurred in the inoculated testis.

In none of the animals which had not been given acacia did lesions develop in the heart.

EXPLANATION OF FIGURE 5

A, scattered necrotic and calcified muscle fibers with accompanying leukocytes in the wall of the right ventricle of the heart of rabbit C8, which was killed seven days after being inoculated intratesticularly and subcutaneously with the virus of inflammatory fibroma. Hematoxylin and eosin; × 122.

B, edema, atrophy of muscle fibers and infiltration by mononuclear and polymorphonuclear leukocytes in the wall of the right auricle of the heart of rabbit C4, which was killed seven days after being inoculated intratesticularly with the virus of inflammatory fibroma. Hematoxylin and eosin; × 122.

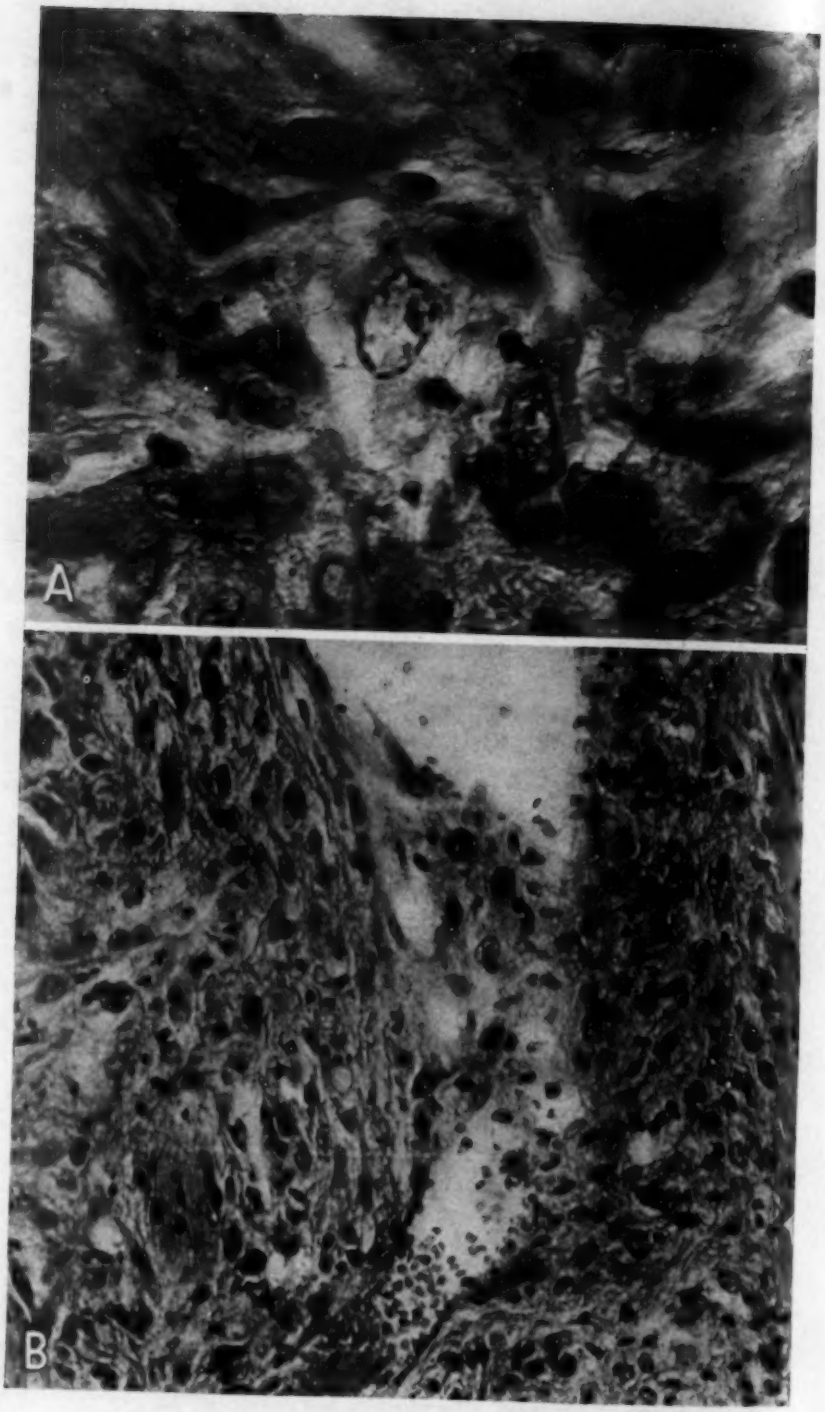

Figure 6

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COMMENT

From these experiments it appears that the ability to localize in the heart is by no means an attribute only of virus III but is common to all of the other viruses of widely differing nature which were studied: vaccine virus, myxoma virus, the two strains of fibroma virus and pseudorabies virus.

TABLE 2.—*Distribution and Severity of Lesions in Hearts of Rabbits Which Were Infected by Peripheral Inoculation of Several Viruses*

Virus	Rabbit	Days After Inoculation	Myocardium	Endocardium	Valves	Pericardium
Vaccine virus	A1	3*	++++	++	0	0
	A2	4*	++	+	+	0
	A3	3	+	0	0	0
	A4	4	+++	0	+++	0
	A5	4*	++	0	+++	0
	A6	4*	++++	0	+++	0
	A7	3*	++	0	+++	0
	A8†	3	+	0	0	0
Pseudorabies virus	B1	3*	+++	+	0	++
	B2	3*	++	0	0	0
	B3	3*	+	+	+	+
	B4	3*	0	0	+++	0
	B5	3*	++++	0	0	0
	B6	3*	++	+	0	0
	B7	3*	++	+	++	+
	B8†	3*	+	+	+	0
	B9†	3*	+	+	+	0
Fibroma virus (inflammatory strain)	C1	6	++	0	0	0
	C2	7	++	+	+	0
	C3	7	+++	0	0	0
	C4	7	+++	+	0	+
	C5	7	+++	0	+	++
	C6	6	+	0	0	0
	C7	5	++++	++	++++	+
	C8	7	++++	0	0	0
	C9†	6	++	+	+	0
Myxoma virus	D1	7	+++	0	0	0
	D2	7*	++++	+++	0	+
	D3	7*	++++	+++	0	++
	D4	9*	++	0	0	0
	D5	5*	++	0	0	0
	D6	7*	++++	++	0	++
	D7†	7	++	++	0	0
	D8†	7	+	0	0	0
	D9†	6*	+	0	0	0
Fibroma virus (strain A)	E1	4	++++	+	0	0
	E2	10	+	0	0	0
	E3	7	+	0	0	0
	E4	7	++++	+	0	+
	E5	9	+	0	0	0
	E6	13	++	0	0	0
	E7	11	+	0	0	0

* These animals were not killed but died as a result of their virus infection.
† Control animals which had not been given a solution of acacia.

EXPLANATION OF FIGURE 6

A, myxoma cells and polymorphonuclear leukocytes in the myocardium of rabbit D2. Hematoxylin and eosin; × 945.

B, thickening of the endocardium by the proliferating myxoma cells and beginning obliteration of an endothelial crevice in the heart of rabbit D3, which died seven days after being inoculated intratesticularly and subcutaneously with the virus of myxoma. Leukocytes and indeterminate nuclear fragments are abundant. Hematoxylin and eosin; × 729.

The localization in the heart, however, is directly influenced by the preliminary intravenous injection of a solution of acacia, and without this preparatory treatment lesions either do not occur in the heart or are of minor intensity. In the work with virus III [1] the intravenous injection of pitressin and the cardiac puncture were also successful methods of preparation. It seems highly probable that these methods would be equally effective in conjunction with the viruses used in the present study. Presumably all produce some alteration, although it may be small and transient, in the anatomic or physiologic state of the heart which makes it more susceptible to infection by the circulating virus which has entered the blood stream from a focus at the site of inoculation. A more precise explanation of the mechanism by which these factors

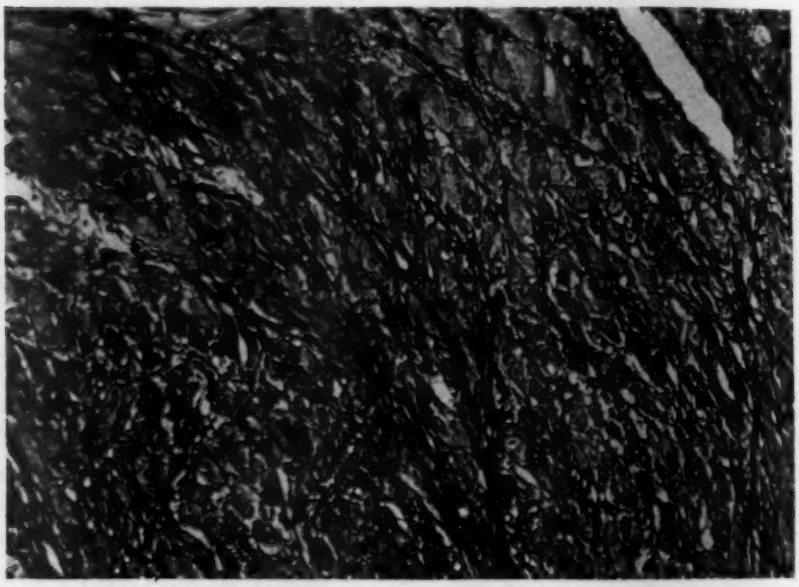

Fig. 7.—Interstitial fibrosis of the myocardium of the right ventricle of the heart of rabbit E4, which was killed seven days after being inoculated intratesticularly and subcutaneously with strain A fibroma virus. Masson's trichrome stain; × 122.

operate to induce the virus to infect the heart selectively is the object of further study.

As in the previous experiments with virus III, the myocardium of the right ventricle, especially in the papillary muscles, was the commonest site of lesions in the animals treated by intravenous injection of a solution of acacia. A possible explanation of this is that the right side of the heart is much more greatly affected than the left by the increased work which the heart must do to accommodate the circulation to the sudden addition to the venous blood of a large volume of a hypertonic solution. The lesions in the hearts of rabbits which had not had

acacia were frequently in the apical portion of the left ventricular musculature. It may be that this part of the heart wall is subject normally to greater strain and is hence more susceptible to the infectious agent.

In this series of experiments there was no significant difference in the incidence of the cardiac involvement produced by the several viruses. There is some indication, however, that the vaccine virus, the myxoma virus and the virus of inflammatory fibroma caused more extensive damage than the strain A fibroma or the pseudorabies virus. It is difficult to relate this observation to virulence since myxoma infection and pseudorabies are invariably fatal, while inflammatory fibroma seldom if ever kills its host. Possibly the severity of the pseudorabic myocarditis is limited by the rapidity with which the animal succumbs to his systemic infection and might be greater if the disease as a whole were less fulminating and allowed a longer time for lesions to mature.

Valvulitis was greatest in vaccinia, less frequent and less extensive in pseudorabies and in the inflammatory fibroma infection and absent in the myxoma and strain A fibroma infections. Although again no conclusions can be drawn from this small series, it might be pointed out that the three viruses which caused inflammation of the valves are those which characteristically induce necrosis and exudation rather than proliferation, and conversely the two which left the valves unchanged are those which characteristically stimulate proliferation and not destruction of tissue.

SUMMARY

The intratesticular inoculation of vaccine, pseudorabies, inflammatory fibroma, strain A fibroma and myxoma viruses into rabbits which had been prepared by a preceding intravenous injection of a solution of acacia was followed by the appearance of cardiac lesions in the majority of the animals. Cardiac lesions did not occur or were of minor intensity in animals which had not had a preceding intravenous injection of the solution of acacia. In this respect the action of these viruses in localizing in the heart is similar to that previously described for virus III.

The lesions, regardless of the specific etiologic agent, were situated predominantly in the myocardium, but the viruses which are more prone to engender necrosis and exudation, i. e., vaccine virus and the viruses of pseudorabies and inflammatory fibroma, occasionally produced inflammation of the auriculoventricular valves.

The reaction in the heart was as a rule typical of the agent causing it. Thus in the acute exudative lesion of pseudorabies the intranuclear inclusion bodies were seen. The myxoma virus induced proliferation of the typical large myxomatous cells, and the fibroma virus that of the characteristic fibroblasts, in the interstices of the heart muscle. The vaccine and inflammatory fibroma viruses brought about a less specific picture of muscle necrosis and leukocytic exudation.

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EFFECT OF THYROXIN AND THE ANTERIOR PITUITARY GROWTH HORMONE ON ENDOCHONDRAL OSSIFICATION

SPECIES USED: THE RAT

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It has long been established that both the thyroid gland and the pituitary gland are related to skeletal growth and differentiation. However, it is still a moot point whether the actions of these two glands are independent or interdependent. Hypophysectomy is followed by cessation of skeletal growth (Aschner [1]; Dott and Frasier [2]; Freud, Levie and Kroon [3]), but in addition the operation causes atrophy of the thyroid gland. Thyroidectomy is followed by a marked decrease if not cessation of skeletal growth (Hammett [4]), but at the same time there is definite atrophy of the acidophil cells of the pituitary gland (Severinghaus, Smelser and Clark [5]). Injections of anterior pituitary extracts are followed by skeletal growth in dogs and cats (Dott and Frasier [1]), rats (Evans and Long [6]) and guinea pigs (Silberberg [7]); or they may be followed by early skeletal maturity (Silberberg and Silberberg [8]). Injections of anterior pituitary extracts also are followed by changes in the

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thyroid gland (Loeb and co-workers [9]). However, at least one fraction of the anterior lobe of the pituitary gland will cause growth in the absence of the thyroid gland (Silberberg [10]). The data available on the action of thyroxin on the skeleton are less definite (Silberberg and Silberberg [11]; Riddle [12]). Finally, synergism has been demonstrated between the thyroid hormone and the growth-promoting fraction of the anterior lobe of the pituitary gland (Evans, Simpson and Pencharz [13]).

The purpose of the investigation reported here was to study the effect of thyroxin and of an anterior pituitary extract containing the growth-promoting hormone and of combinations of the two on the skeletons of normal female rats, thyroparathyroidectomized female rats and thyroparathyroidectomized-hypophysectomized female rats.

MATERIAL AND METHODS

The rats for this study, which was a part of an experiment previously reported (Evans, Simpson and Pencharz [13]), were divided into three series (table 1): (1) normal females not operated on, (2) thyroparathyroidectomized females and (3) thyroparathyroidectomized-hypophysectomized females.

The normal rats not operated on were subdivided into four groups: (*a*) untreated controls; (*b*) animals given a daily injection of 0.005 mg. of crystalline thyroxin, a dose which was found sufficient to establish a normal basal metabolic rate in the thyroparathyroidectomized rats; (*c*) animals given a daily injection of 1 cc. of a standard alkaline extract of bovine anterior pituitary lobe containing 15 to 17 mg. of organic material; animals given injections of both thyroxin and anterior pituitary extract at the same levels as the aforementioned groups.

The thyroparathyroidectomies were performed when the rats had reached 35 to 45 days of age. To determine the completeness of the operations, the basal metabolic rate of each rat was taken after a postoperative period of approximately three months. No animal was used whose rate of oxygen consumption was more than 130 liters of oxygen per square millimeter per twenty-four hours (normal 150). The animals finally chosen were subdivided into four groups and treated the same as the normal animals (table 1, series 2).

For the last series, a number of the thyroparathyroidectomized animals were also hypophysectomized. One day after operation these were subdivided into four groups which received the same treatment as the normal and the thyroparathyroidectomized animals. Unfortunately, because of the mortality of the animals doubly operated on, during the prolonged experimental period, only survivors of two groups were available for study, the group given injections of thyroxin (table 1, series 3, group *a*) and the group given injections of thyroxin and anterior pituitary extract (group *b*).

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9. Loeb, L., and Bassett, R. B.: Proc. Soc. Exper. Biol. & Med. **26**:860, 1929. Loeb, L., and Friedman, H.: ibid. **29**:172, 1931.

10. Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **33**:554, 1936.

11. Silberberg, M., and Silberberg, R.: Growth **2**:327, 1939.

12. Riddle, cited by Evans, Simpson and Pencharz.[13]

13. Evans, H. M.; Simpson, M. E., and Pencharz, R. I.: Endocrinology **25**: 175, 1939.

TABLE 1.—*Data on the Three Series of Rats Used in the Investigation*

Series and Group	Rat	1 Age at Operation, Days	2 Age at Onset of Injections, Days	3 Age at Autopsy, Days	4 Body Weight at Autopsy, Days
1. Rats not operated on:					
(a) Controls.....	1	..	...	305	304
	2	..	...	305	250
	3	..	...	365	294
	4	..	...	365	304
	5	..	...	374	286
	6	..	...	384	314
	7	..	...	384	250
	8	..	...	406	268
	9	..	...	413	286
	10	..	...	428	290
	11	..	...	435	340
	12	..	...	443	332
	13	..	...	449	272
	14	..	...	450	300
	Average	..	...	...	292
(b) Thyroxin-treated.....	1	..	51	302	262
	2	..	...	302	334
	3	..	...	302	254
	4	..	...	302	274
	5	..	...	302	246
	6	..	...	302	240
	Average	..	...	...	268
(c) Treated with anterior pituitary extract containing the growth hormone	1	..	54	305	514
	2	..	...	...	384
	3	..	...	...	452
	4	..	...	...	454
	5	..	...	...	390
	Average	..	...	...	439
(d) Treated with both thyroxin and extract	1	..	54	305	526
	2	..	...	...	504
	3	..	...	...	460
	4	..	...	...	512
	5	..	...	...	404
	6	..	...	...	544
	Average	..	...	...	492
2. Thyroparathyroidectomized rats:					
(a) Controls.....	1	35	...	365	154
	2	35	...	365	134
	3	42	...	383	167
	4	42	...	383	182
	5	37	...	402	145
	6	38	...	451	153
	Average	..	...	...	156
(b) Thyroxin-treated.....	1	37	106	405	226
	2	43	85	383	252
	3	48	121	372	232
	4	35	174	435	244
	5	60	201	462	286
	6	35	108	364	332
	Average	..	...	...	262
(c) Treated with anterior pituitary extract containing the growth hormone	1	42	123	385	442
	2	35	108	330	332
	3	38	188	450	334
	4	39	190	413	300
	Average	..	...	...	352
(d) Treated with both thyroxin and extract	1	35	108	364	502
	2	43	123	384	472
	3	35	108	364	416
	4	38	181	442	596
	5	42	123	384	466
	Average	..	...	...	502
3. Thyroparathyroidectomized-hypophysectomized rats:					
(a) Thyroxin-treated.....	1	57- 99	100	406	168
	2	35-128	129	434	148
	Average	..	...	...	158
(b) Treated with both thyroxin and anterior pituitary extract containing growth hormone...	1	38-121	122	427	470

The right tibia, the costochondral junction of the third right rib and the skull (to be reported on in a subsequent paper) were fixed in Zenker-formaldehyde solution,[14] decalcified in an alcoholic solution of nitric acid, dehydrated, embedded in nitrocellulose and serially sectioned at 8 microns. Three staining procedures were employed: Böhmer's hematoxylin and eosin, Mallory-azan and Koneff's [15] iron hematoxylin–aniline blue–methyl green. Only sections through the central portion of the lateral articular surface of the tibia (proximal epiphysis) and only central sections of the rib were used for comparative purposes. Measurements were made with a micrometer ocular that had been standardized against a calibrated slide. The cortical bone of the tibia was measured in the center of the shaft on the posterior surface, the articular cartilage in the central portion of the articular surface and the epiphysial cartilage in the center of the disk. Measurements of the ribs are self explanatory except for "reorganized cartilage." This term refers to that portion of the costochondral junction that corresponds to the epiphysial disk of the tibia. It is clearly shown in the figures.

RESULTS

Series 1. Rats Not Operated on.—The skeletal measurements of the normal rats are presented in table 2.

(*a*) Controls: The normal rat tibia and particularly the epiphysial disk has been described from the standpoint of histology by several investigators; Ray, Evans and Becks [16]). The costochondral junction of the rib is similar in all respects but one to the tibia. Instead of an epiphysial disk there is an area of "reorganized cartilage," in which the various zones—embryonic, basophilic, vesicular and erosive—may be distinguished.

(*b*) Rats Given Injections of Thyroxin: A comparison of the average body weight of the normal rats treated with thyroxin, 268 Gm., with that of the control group, 292 Gm. (table 1, series 1 [*b* and *a*]), indicates that there has been some retardation in weight increase because of the injections. The average body length of the treated animals (table 2 [*b*]), nose-anus 22.0 cm. and anus-tail 18.6 cm., shows no stimulation of vertebral growth over the normal (nose-anus 22.2 cm., anus-tail 18.7 cm.). The slight increase in average cortical bone width of the tibia (5 units, table 2) and of the rib (1 unit) over the normal is difficult to interpret because of the wide variation in the normal. Measurements of the cartilage in both the tibia and the rib (table 2) indicate that thyroxin causes no definite stimulation of this tissue in these animals.

Survey pictures of the tibia (fig. 1 *B*) and the rib show little variation from the normal. However, higher magnifications (figs. 4 *B* and 7 *B*)

14. Zenker-formaldehyde solution is Zenker's solution prepared with 10 per cent neutral solution of formaldehyde U. S. P. instead of acetic acid.

15. The formula and method may be obtained by communicating with Dr. A. Koneff, University of California, Berkeley, Calif.

16. Ray, R. D.; Evans, H. M., and Becks, H.: Am. J. Path. **17**:509, 1941.

indicate a more regular vesicular zone in both the rib and the tibia and some stimulation of ossification, resulting in fewer islands of cartilage in the shaft and somewhat more abundant cancellous bone.

TABLE 2.—*Skeletal Measurements for Series 1 (Normal Female Rats Not Operated on)*

		Body Length at Autopsy		Tibia, Proximal End			Third Rib, Costochondral Junction		
		1	2	3	4	5	6	7	8
Group	Rat	Nose to Anus, Cm.	Anus to Tail, Cm.	Cortical Bone Width*	Articular Cartilage Width*	Epiphysial Cartilage Width*	Total Width*	Cortical Bone Width*	Reorganized Cartilage Width*
(*a*) Controls.....	1	22.25	18.6	..	..	..	77	7	47
	2	21.0	18.9	18	..	28	72	7	39
	3	22.1	19.4	..	..	..	66	6	28
	4	22.5	18.7						
	5	22.8	18.0						
	6	22.3	18.1						
	7	21.0	17.3	28	15	25	50	6	31
	8	21.8	18.5	19	14	23	65	6	28
	9	21.5	17.6						
	10	24.2	20.2						
	11	23.0	20.2						
	12	22.5	19.9						
	13	21.3	18.6						
	14	22.2	18.6						
	Average	22.2	18.7	22	15	25	66	6	35
(*b*) Thyroxin treated...	1	22.3	19.6	29	16	20	65	8	43
	2	22.9	18.7	26	14	20	70	7	16
	3	20.8	17.5	..	..	..	72	8	35
	4	22.4	19.2	..	..	..	50	5	39
	5	22.3	18.4						
	6	21.2	18.3	27	14	22	67	6	28
	Average	22.0	18.6	27	15	21	65	7	32
(*c*) Treated with anterior pituitary extract containing the growth hormone	1	25.7	21.3	25	23	34	92	11	71
	2	23.8	20.4	19	..	25	85	5	59
	3	25.0	20.0	21	28	30	73	11	59
	4	24.5	21.2	31	10	32			
	5	23.8	19.4	25	21	33			
	Average	24.6	20.5	24	21	31	83	9	63
(*d*) Treated with both thyroxin and extract	1	22.6	22.3	22	17	25	72	11	59
	2	26.2	22.3	..	..	..	80	10	55
	3	24.2	21.6	38	22	23	86	11	51
	4	25.6	20.0	25	16	29	82	8	47
	5	24.5	21.2	..	16	30	54	7	47
	6	25.9	21.6	..	..	..	87	9	43
	Average	24.8	21.5	28	18	27	77	9	50

* The width was measured with a calibrated micrometer ocular.

(*c*) Rats Given Injections of an Anterior Pituitary Extract Containing the Growth Hormone: In contrast to the previous group, the rats given injections of an anterior pituitary extract containing the growth-

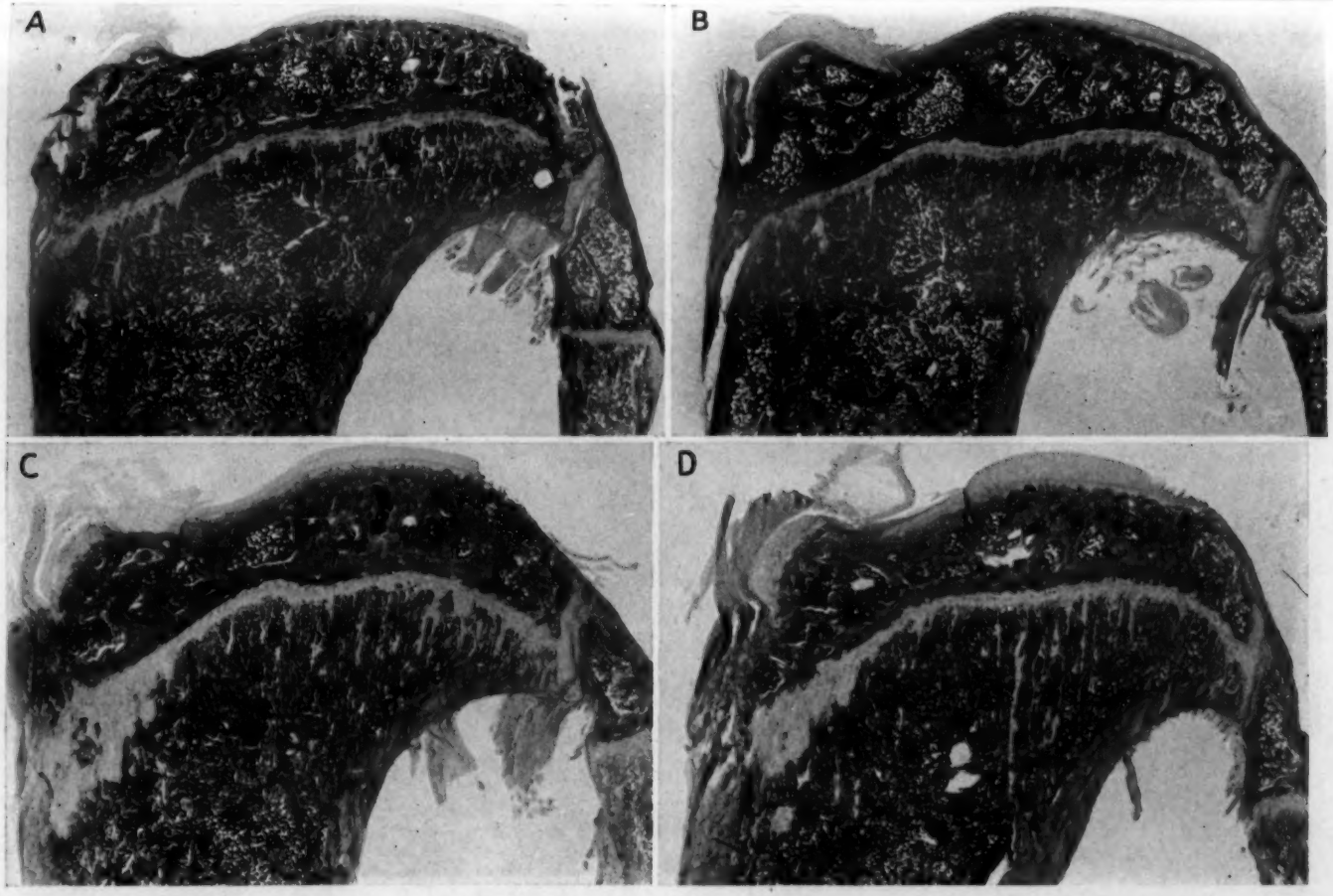

Fig. 1.—Proximal epiphysis of the tibia in series 1 (female rats not operated on): *A*, control; *B*, rat given injections of thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and pituitary extract.

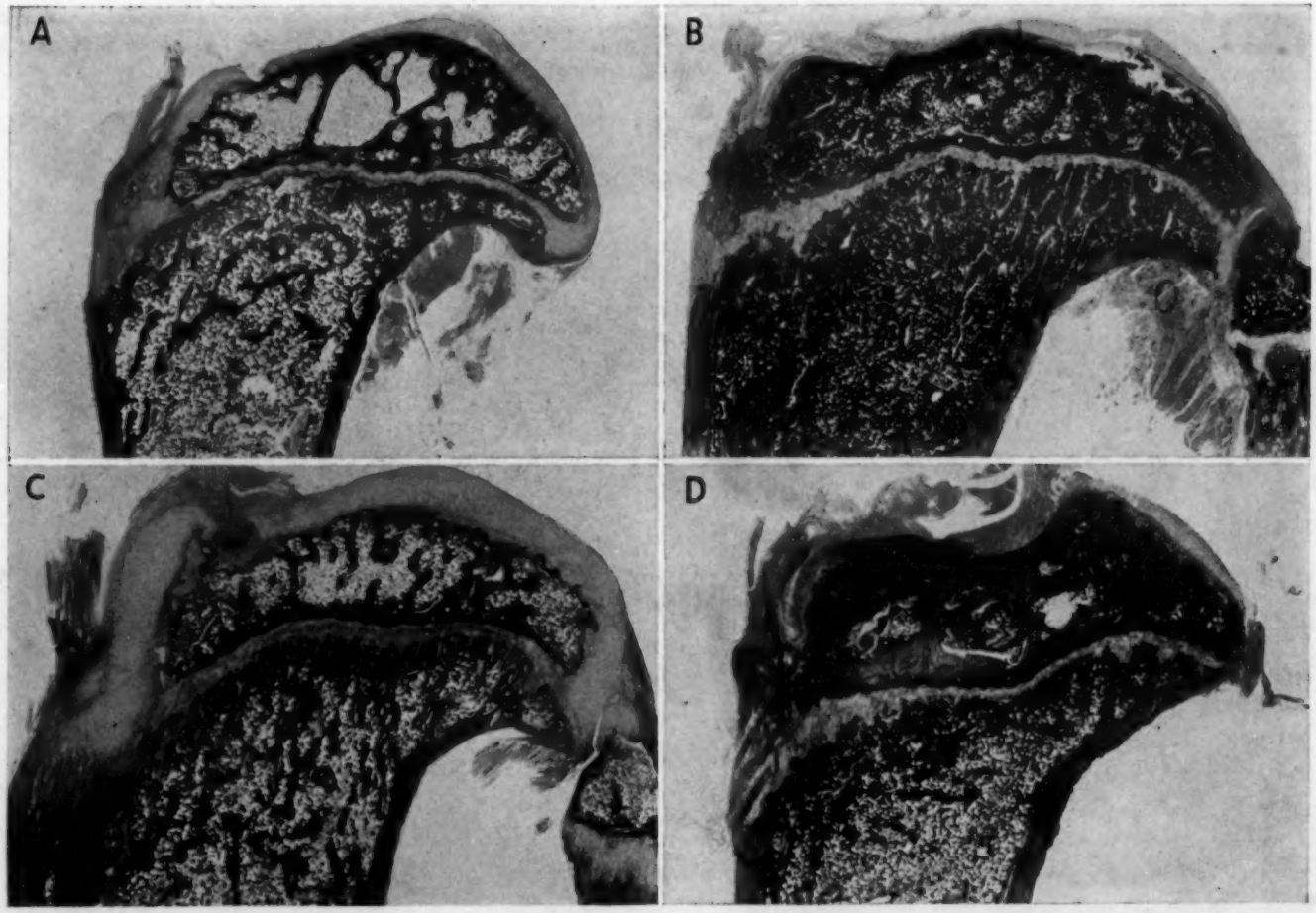

Fig. 2.—Proximal epiphysis of the tibia in series 2 (thyroparathyroidectomized female rats): *A*, control; *B*, rat given injections of thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and extract.

promoting hormone showed a marked response in body weight, the average being 439 Gm., compared with 292 Gm. for the controls (table 1, series 1 [*c* and *a*]). The average body length was also greater—nose-

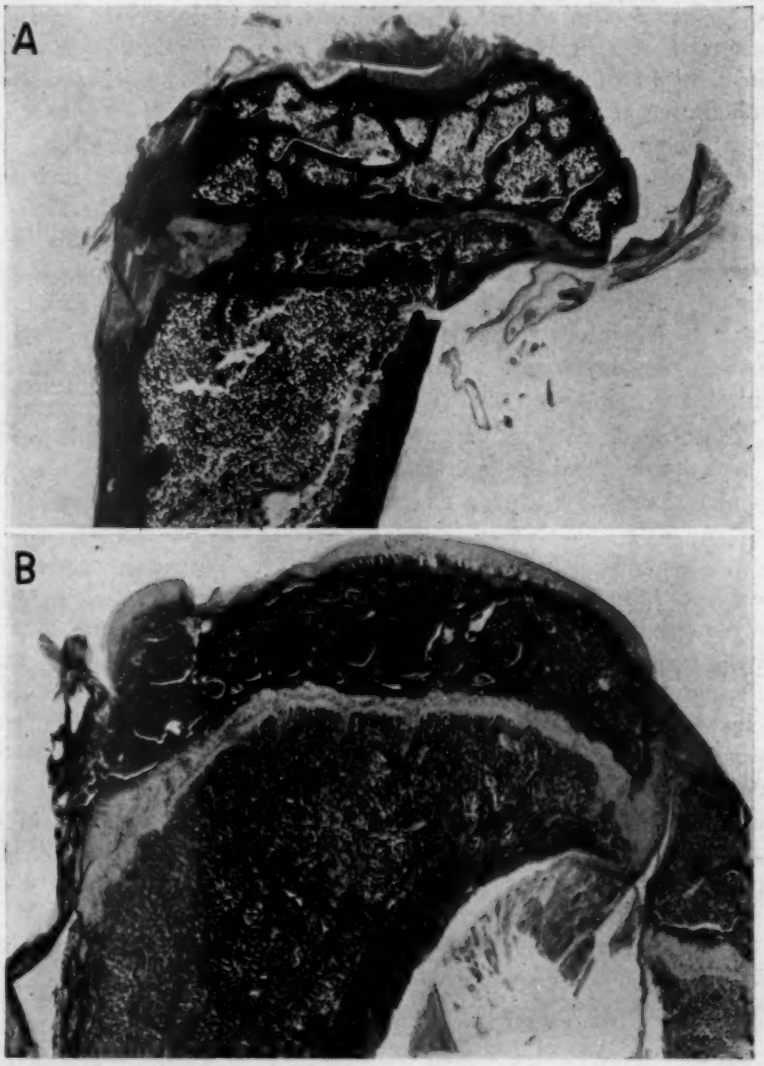

Fig. 3.—Proximal epiphysis of the tibia in series 3 (thyroparathyroidectomized-hypophysectomized female rats): *A*, rats given injections of thyroxin; *B*, rats given injections of both thyroxin and an anterior pituitary extract containing the growth hormone.

anus 24.6 cm., compared with 22.2 cm. (table 2 [*c* and *a*]) and anus-tail 20.5 cm., compared with 18.7 cm. The increase in thickness of the

cortical bone (table 2, columns 3 and 7) is irregular and inconclusive. Measurements of the cartilage (table 2, columns 4, 5, 6 and 8) indicate a certain degree of hypertrophy [17] in both the tibia and the rib.

Histologic examination showed the extent of the cartilaginous hypertrophy more clearly than the averages referred to in the foregoing paragraph. The increase of cartilaginous matrix was seen to be accompanied with an increase in the size and the number of the basophilic or proliferating cells as shown in figures 4 *C* and 7 *C*. The figures also show a definite vesicular zone. Slender columns of cartilage extend deep into the diaphysis of the tibia, and along these bone formation is very active. In numerous places along the epiphysial disk of the tibia and the reorganized cartilage of the rib, as well as in isolated areas in the marrow cavities of both bones, peculiar areas of homogeneous, noncellular, faintly eosinophilic matrix were found—shown in figure 4 *C*. These areas are characteristic of the rats treated chronically with the anterior pituitary extract, although they may also be found on rare occasions in animals given thyroxin.

(*d*) Rats Given Injections of Both Thyroxin and Extract: Injections of both thyroxin and the anterior pituitary extract containing the growth hormone have a synergistic effect on the body weight. Whereas the average for the animals treated with the extract was 439 Gm., the average for this group is 492 Gm. (table 1 [*c* and *d*]). The difference between the effect of the combined substances and that of the extract alone on the body length is less striking: nose-anus 24.8 cm., compared with 24.6 cm. for the extract alone, and anus-tail 21.5 cm., compared with 20.5 cm., a difference in total body length of only 1.2 cm. (table 2 [*d* and *c*]). The increase in thickness of the cortical bone of the tibia and the rib over normal is a little greater than for thyroxin alone (table 2 [*d* and *b*]), although the difference is again complicated by the wide range of individual measurements. The average thickness of the cartilage in the various regions (table 2, columns 4, 5, 6 and 8) strikes a balance between the hypertrophy obtained in the rats treated with the extract and the lack of response in those treated with thyroxin.

Histologically, the changes in the tibia and the rib shown in figures 1 *D*, 4 *D* and 7 *D* are what would be expected on the basis of the aforementioned measurements.

Series 2. Thyroparathyroidectomized Rats.—(*a*) Controls: As might be expected on the basis of clinical data, thyroparathyroidectomy is

17. The question arises as to whether the term "hypertrophy" (hyper + trophe, Greek word for nutrition) has been misused in the text, i. e., whether the increase in the amount of matrix substance has not come about through increased efficiency on the part of the cells rather than increased nutrition by way of the circulation. No evidence was present to indicate the latter.

followed by dwarfing both in body weight, 156 Gm. on the average, compared with 292 Gm. (table 1, series 1 [group *a*] and 2 [group *a*]), and in body length, nose-anus average 17.6 cm., against 22.2 for the normal, and anus-tail average 13.8 cm., compared with 18.7 cm. (columns 1 and 2 in table 3 [*a*] and table 2 [*a*]). There is a decrease in the average width of the cortical bone in the tibia, 13 units, compared with 22 (column 3 in table 3 [*a*] and table 2 [*a*]), but in the rib it is normal in thickness

TABLE 3.—*Skeletal Measurements for Series 2 (Thyroparathyroidectomized Female Rats)*

		Body Length at Autopsy		Tibia, Proximal End			Third Rib, Costochondral Junction		
		1	2	3	4	5	6	7	8
Group	Rat	Nose to Anus, Cm.	Anus to Tail, Cm.	Cortical Bone Width*	Articular Cartilage Width*	Epiphysial Cartilage Width*	Total Width*	Cortical Bone Width*	Reorganized Cartilage Width*
(*a*) Controls.....	1	17.5	13.6						
	2	16.7	12.7	..	..	..	70	8	47
	3	17.5	14.2	12	14	26	56	5	24
	4	18.5	15.0	..	..	..	55	7	39
	5	17.5	13.0	..	..	..	50	7	35
	6	17.8	14.3	14	11	22	52	5	39
	Average	17.6	13.8	13	13	24	57	6	36
(*b*) Thyroxin-treated...	1	20.2	17.2	20	17	23	64	9	39
	2	21.3	18.6	22	17	30	71	10	31
	3	21.0	16.5	15	15	30	65	6	20
	4	21.0	16.5						
	5	20.1	18.1						
	6	22.0	17.9	..	..	..	82	6	39
	Average	20.9	17.5	19	16	26	71	8	32
(*c*) Treated with anterior pituitary extract containing the growth hormone	1	21.0	18.1	..	..	..	82	10	43
	2	20.4	16.5	16	29	36			
	3	20.8	16.6	19	30	40	88	7	39
	4	20.5	17.1	..	..	..	74	10	51
	Average	20.7	17.1	18	30	38	81	9	44
(*d*) Treated with both thyroxin and extract	1	25.5	21.2	28	23	27	74	10	47
	2	24.5	20.0	20	17	22	84	8	31
	3	24.1	20.4	26	23	28	82	7	47
	4	25.6	18.3	30	28	23			
	5	24.5	20.5						
	Average	24.8	20.1	26	23	25	80	8	42

* The width was measured with a calibrated micrometer ocular.

(column 7 in table 3 [*a*] and table 2 [*a*]). The articular cartilage (column 4), the epiphysial cartilage of the tibia (column 5) and the reorganized cartilage of the rib (column 8) are normal in thickness. The average width of the costochondral junction is less than normal (column 6).

Histologically, the tibia (table 3 [*a*]) shows in addition to the diminished thickness of the cortical bone a very light formation of cancellous bone in both the epiphysis and the diaphysis. Another striking feature is the tremendous increase in the fat content of the marrow (fig. 2 *A*). The epiphysial cartilage, shown in figure 5 *A*, is very irregular and is capped

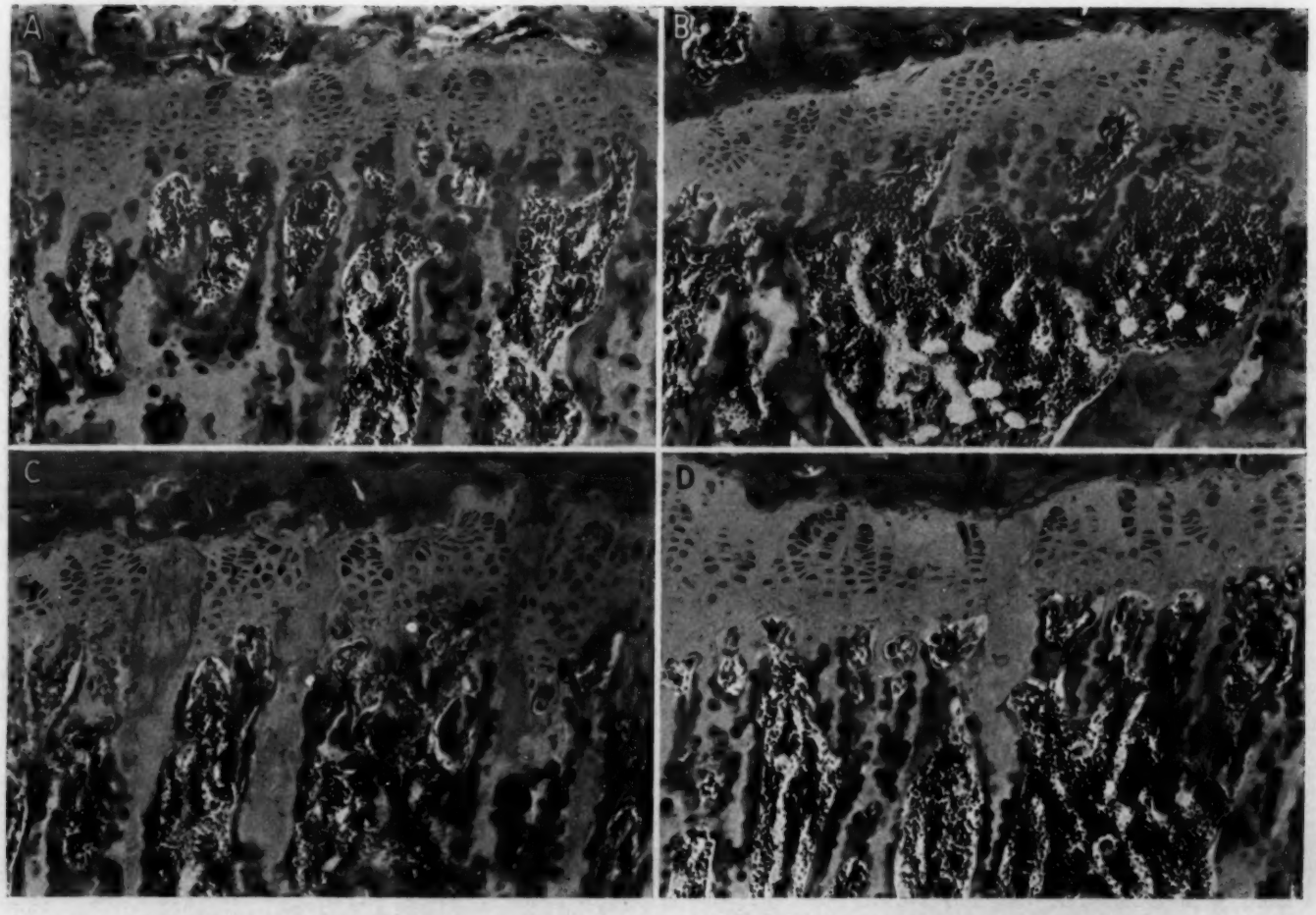

Fig. 4.—Epiphysial disk of tibia in series 1 (female rats not operated on): *A*, control; *B*, rat given injections of thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and extract.

extract.

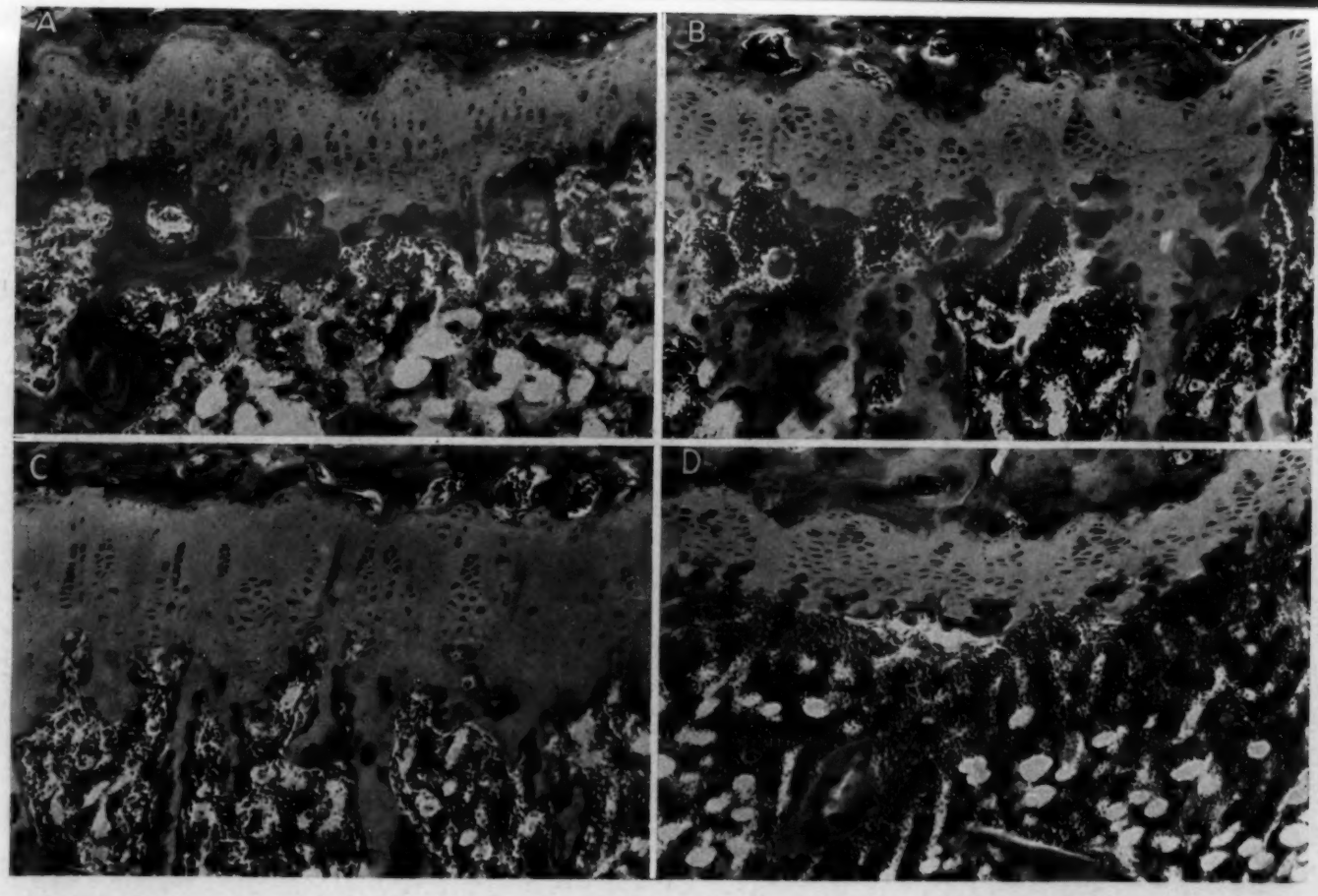

Fig. 5.—Epiphysial disk of tibia in series 2 (thyroparathyroidectomized female rat) : *A*, control; *B*, rat given injections of thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and extract.

on the epiphysial side with a layer of light-staining matrix that resembles the "embryonic" zone in the young rat. These changes are not seen in the rib (fig. 8 *A*).

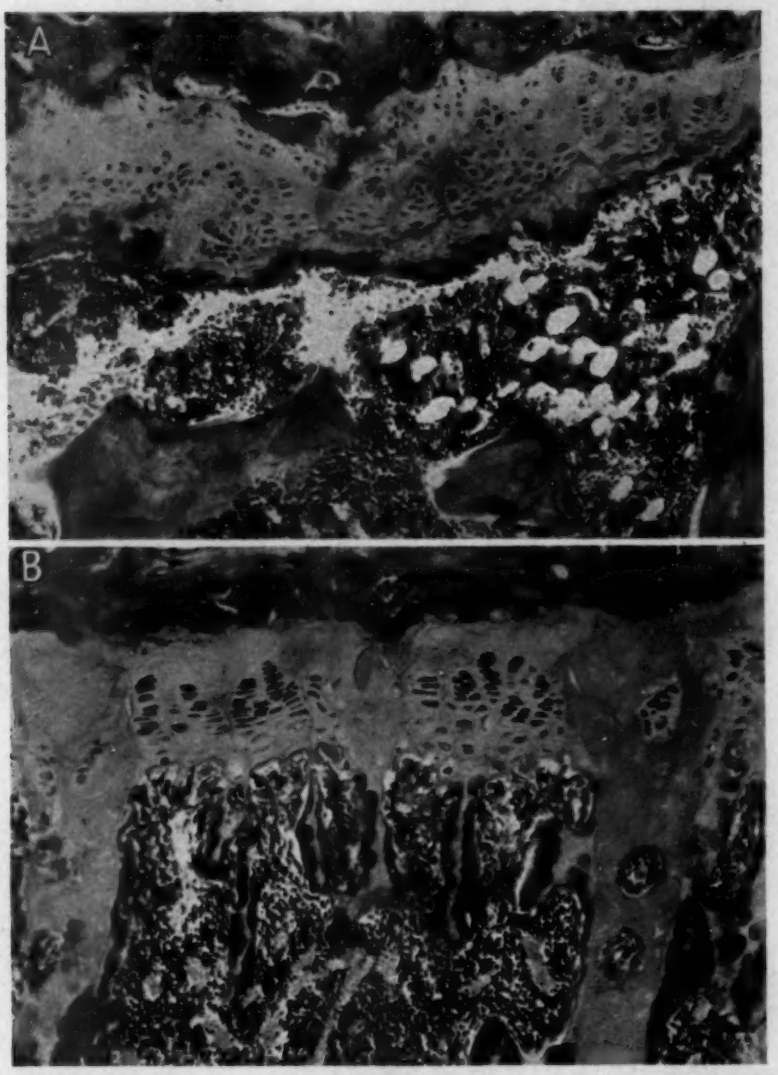

Fig. 6.—Epiphysial disk of tibia in series 3 (thyroparathyroidectomized-hypophysectomized female rats): *A*, rat given injections of thyroxin; *B*, rat given injections of both thyroxin and an anterior pituitary extract containing the growth hormone.

The arrangement of the basophilic cells is seen to be very irregular. The cells are small and the matrix relatively abundant. The vesicular

zone is irregular; the lacunas and the cells occupying them are small. The nuclei of the latter are pyknotic. The erosion zone is replaced by a layer of bone. Changes in the rib—shown in figure 8 *A*—are somewhat different. Here the vesicular zone is more regular, although staining very lightly, and the zone of erosion is still present.

(*b*) Rats Given Injections of Thyroxin: Following injections of thyroxin, the average body weight of the thyroparathyroidectomized rats approaches that of the normal animals, 262 Gm. for the former, 292 Gm. for the latter (table 1, series 2 [*b*] and series 1 [*a*], respectively). The average body length is greater than that of the controls in this series but still less than normal, nose-anus 20.9 cm. and anus-tail 17.5 cm. (table 3 [*b*], columns 1 and 2). However, the cortical bone of the tibia (table 3, column 3) and the rib (column 7) and the cartilage in the various regions (columns 4, 5, 6 and 8) are all normal in width.

The histologic response to the injections is shown in figures 2 *B*, 5 *B* and 8 *B*. Essentially, there is a response in both cartilage and bone formation, with return to the normal configuration and repair of the fatty degeneration of the marrow of the tibia that followed the operation.

(*c*) Rats Given Injections of an Anterior Pituitary Extract Containing the Growth Hormone: The average body weight of the thyroparathyroidectomized rats given an anterior pituitary extract containing the growth hormone, 352 Gm., is considerably greater than that of the animals given thyroxin in this series (table 1, series 2 [*c* and *b*]). However, the average body length, nose-anus 20.7 cm. and anus-tail 17.1 cm., is approximately the same (table 3 [*c* and *b*], columns 1 and 2). The thickness of the cortical bone, in both the tibia and the rib, is slightly greater than that of the controls in this series but still not normal (table 3, columns 3 and 7). The most striking response of this group of animals, however, is the cartilaginous hypertrophy in all regions: articular cartilage 30 units, in contrast to the control average of 13 (table 3, column 4); epiphysial cartilage 38 units, compared with 24 (column 5); total width of the costochondral junction 81 units, compared with 57 for the controls (column 6), and reorganized cartilage 44 units, against 36 for the controls (column 8).

From the histologic standpoint, in addition to the pronounced stimulation of the cartilage there has also been some stimulation of cancellous bone formation as represented by increased numbers of trabeculae in the shaft (fig. 2 *C*). On the other hand, there has been little response in the marrow, for the fat content is still abnormally great. Figure 5 *C* shows the details of the response of the epiphysial cartilage. The whole disk is hypertrophied but regular in width. The cells of the basophilic zone are large and fairly well oriented. The vesicular zone is irregular and lacking in places. The response of the costochondral junction of the

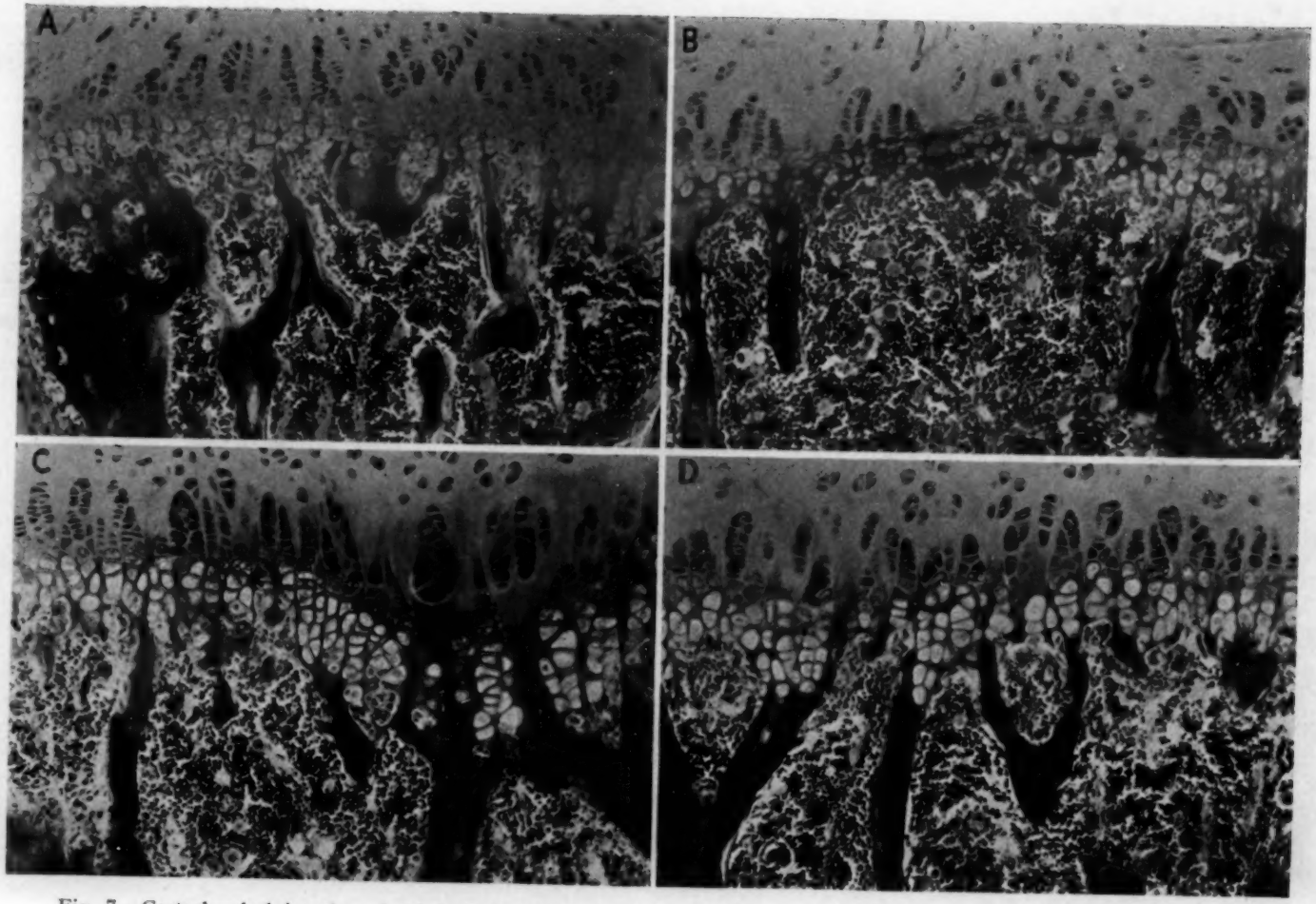

Fig. 7.—Costochondral junction of third rib in series 1 (female rats not operated on): *A*, control; *B*, rat given injections of thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and extract.

rib, shown in figure 8 *C*, was equally marked both in the reorganized cartilage and in the trabecular formation. The noncellular areas of matrix described previously are also present in these animals.

(*d*) Rats Given Injections of Both Thyroxin and Extract: As in the series of rats not operated on, the combined injections of thyroxin and anterior pituitary growth hormone in the thyroparathyroidectomized animals act to produce a greater average body weight than either substance produces alone: 502 Gm. for this group, 352 Gm. for the group treated with the extract, 262 Gm. for the group treated with thyroxin (table 1, series 2, groups *d*, *c* and *b*, respectively). The two hormones also act synergistically to some extent on vertebral growth, the average nose-anus length of rats given injections of both thyroxin and the extract being 24.8 cm., compared with 20.7 cm. average for those treated with the extract (table 3, column 1), and the average anus-tail length being 20.1 cm., compared with 17.1 cm. (column 2). There is considerable variation in the thickness of the cortical bone of the tibia, 20 to 30 units, the average of 26 being somewhat greater than the control of 13 units (table 3, column 3); the cortical bone of the rib is less responsive, 8 units average width, compared with 6 (column 7); cartilaginous hypertrophy is not as marked in this group as in animals given the extract alone, as indicated by the measurements of the various regions (table 3 [*d* and *c*], columns 4, 5, 6 and 8).

Histologically the tibia (fig. 2 *D*) shows a definite increase in the amount of bone in the epiphysis and an increase in the number of cells of the epiphysial disk (fig. 5 *D*). The latter, however, is not accompanied by a corresponding increase in matrix but rather by stimulation of bone formation in the shaft. The vesicular zone is very narrow and in places lacking altogether. The response of the rib (fig. 8 *D*) differs from the tibia in that both the basophilic zone and the vesicular zone are stimulated. Cancellous bone is more abundant than in the group treated with the extract or that treated with thyroxin.

Series 3. Thyroparathyroidectomized-hypophysectomized Rats.—(*a*) Injections of Thyroxin: Unfortunately, control animals for this group are lacking; so the measurements cannot be compared with those of rats similarly operated on but not given injections. The average body weight is 158 Gm. (table 1, series 3 [*a*]), approximately the same as the average for the thyroparathyroidectomized controls (series 2 [*a*]). The average body length, nose-anus 17.5 cm., anus-tail 14.5 cm. (table 4 [*a*], columns 1 and 2) is also approximately the same as for the controls of series 2. The cortical bone of the tibia is somewhat thicker (table 4, column 3) but that of the rib (column 7) is the same in both groups. The articular cartilage is not as thick as in the thyroparathyroidectomized controls (column 4), but the epiphysial disk (column 5), costochondral junction (column 6) and reorganized cartilage (column 8) are approximately the same in average width.

thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and extract.

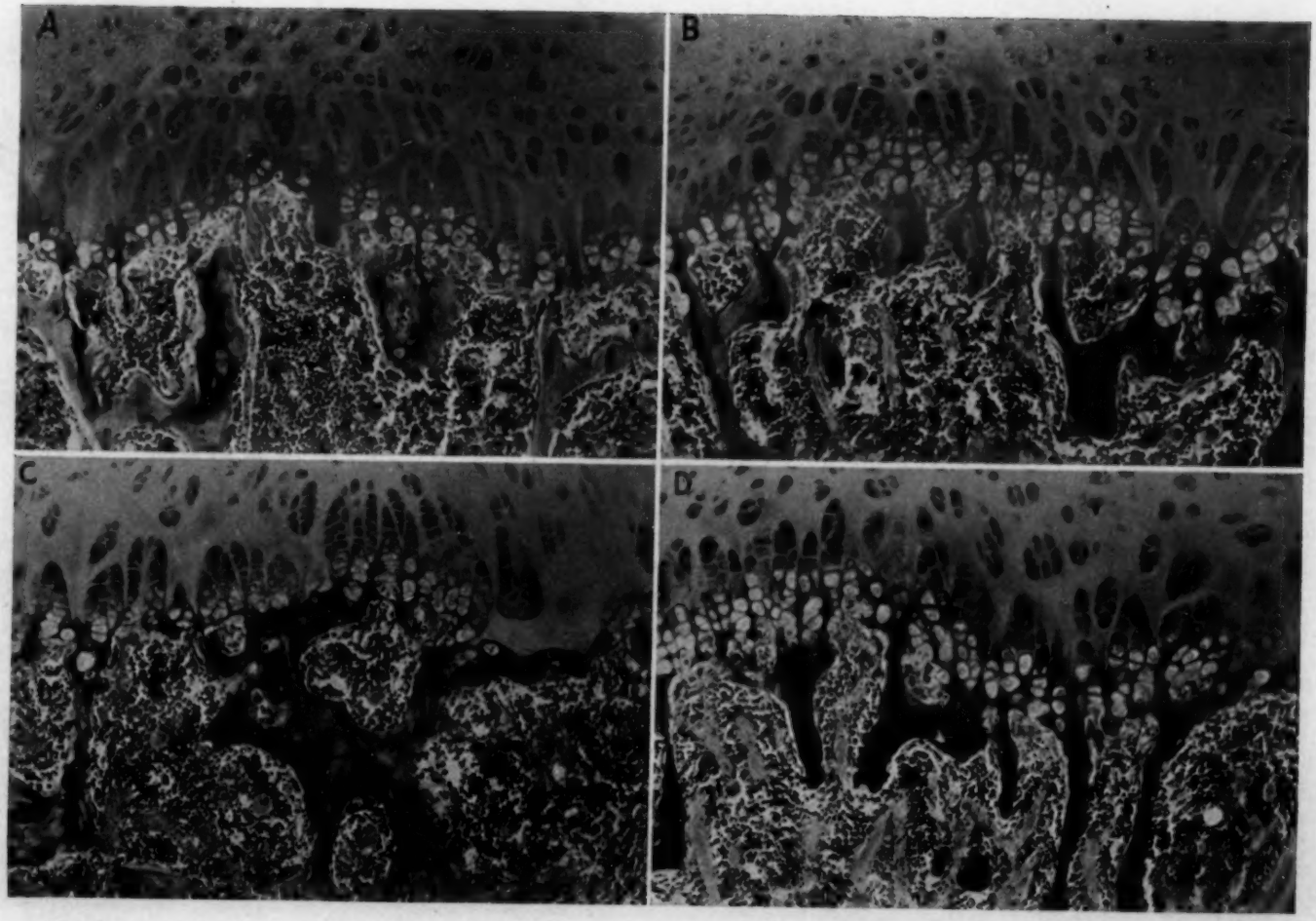

Fig. 8.—Costochondral junction of third rib in series 2 (thyroparathyroidectomized female rats): *A*, control; *B*, rat given injections of thyroxin; *C*, rat given injections of an anterior pituitary extract containing the growth hormone; *D*, rat given injections of both thyroxin and extract.

Histologically, the tibia changes are similar to those in the thyroparathyroidectomized control, but they are considerably more pronounced. Reference to figure 3 *A* will show that the resorption of cancellous bone

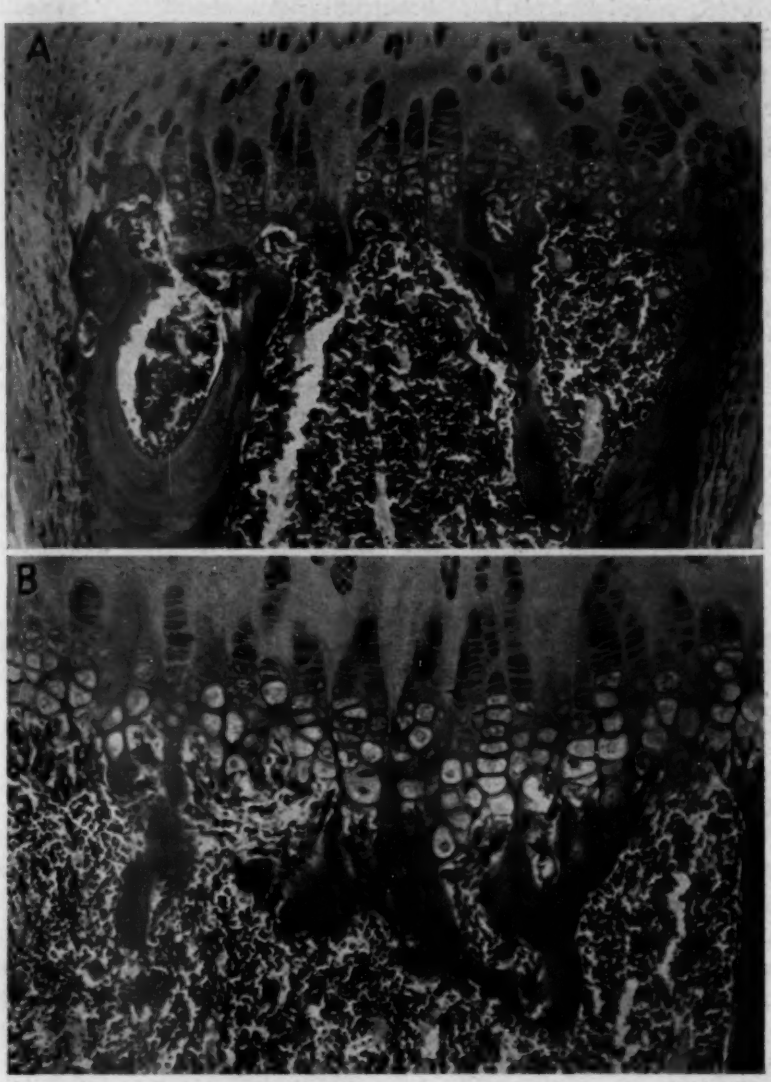

Fig. 9.—Costochondral junction of third rib in series 3 (thyroparathyroidectomized-hypophysectomized female rats): *A*, rat given injections of thyroxin; *B*, rat given injections of both thyroxin and an anterior pituitary extract containing the growth hormone.

and the fatty degeneration of the marrow are marked; the trabeculae that remain are heavy and completely ossified; the epiphysial cartilage is

irregular in width, and in several places it is broken through by bone formation. Figure 6 *A* shows the basophilic cells to be numerous and very irregularly arranged. They are larger than in the thyroparathyroidectomized controls, but the matrix is not as abundant. A vesicular zone is absent, and the zone of erosion is replaced by bone. The changes in the rib are shown in figure 9 *A*. Here, although the basophilic cells are well oriented and the vesicular zone is present, little growth has taken place, as indicated by the size of the costochondral junction.

(*b*) Rats Given Injections of Both Thyroxin and an Anterior Pituitary Extract Containing the Growth Hormone: In contrast to injections of thyroxin alone, combined injections of thyroxin and extract in a rat doubly operated on resulted in a definite increase in body weight,

TABLE 4.—*Skeletal Measurements for Series 3 (Thyroparathyroidectomized-Hypophysectomized Female Rats)*

		Body Length at Autopsy		Tibia, Proximal End			Third Rib, Costochondral Junction		
		1	2	3	4	5	6	7	8
Group	Rat	Nose to Anus, Cm.	Anus to Tail, Cm.	Cortical Bone Width*	Articular Cartilage Width*	Epiphysial Cartilage Width*	Total Width*	Cortical Bone Width*	Reorganized Cartilage Width*
(*a*) Thyroxin-treated...	1	17.5	15	..	..	..	50	6	43
	2	17.4	14.0	20	7	25			
	Average	17.5	14.5						
(*b*) Treated with both thyroxin and extract containing the growth hormone....	1	23.5	21.1	26	14	40	95	11	71

* The width was measured with a calibrated micrometer ocular.

470 Gm. at autopsy as against an average of 158 Gm. (table 1, series 3, column 4). The response in vertebral growth as evidenced by the body length was also pronounced, 23.5 cm. nose-anus length, against 17.5 cm. (table 4, column 1), and 21.1 cm. anus-tail length, compared with 14.5 cm. Other measurements indicate a corresponding stimulation of bone and cartilage: tibial cortical bone width 26 units (table 4, column 3), rib cortical bone 11 units (column 7), articular cartilage of tibia 14 units (column 4), epiphysial cartilage 40 units, costochondral junction width 95 units (column 6) and reorganized cartilage 71 units (column 8).

The tibia (fig. 3 *B*) shows normal distribution of the cancellous bone with numerous small trabeculae in the shaft around which osteogenesis is extremely active. The epiphysial disk (fig. 6 *B*) is wider than normal but regular. In the proliferating zone the cells are large, numerous and well oriented. The vesicular zone is fairly regular in width, and there is definite activity along the zone of erosion. Figure 9 *B* of the costo-

chondral junction of the rib shows changes essentially the same as those in the tibia. A comparison with figures 6*A* and 9*A* of the animal doubly operated on and treated with thyroxin will give some indication of the profound nature of the response.

COMMENT

The process of endochondral ossification is involved in growth in body length, in the long bones and in the ribs. Since this process is initiated in the cartilage, any factors affecting such growth would naturally exert their initial influence there. A stimulation of endochondral

TABLE 5.—*Summary of Histologic Observations at the Zone of Endochondral Ossification in the Tibia and the Rib*

Series and Group	Number of Cells	Size of Cells	Amount of Matrix	Replacement by Bone
1. Normal rats:				
(a) Thyroxin	Normal	Normal	Normal	Increased
(b) Anterior pituitary extract containing the growth hormone	Increased	Increased	Increased	Active
(c) Both	Decreased	Normal	Increased	Increased
2. Thyroparathyroidectomized rats:				
(a) Controls	Normal	Decreased	Normal	Inactive
(b) Thyroxin	Normal	Normal	Normal	Normal
(c) Anterior pituitary extract containing the growth hormone	Decreased	Increased	Markedly increased	Active
(d) Both	Increased	Increased	Decreased	Increased
3. Thyroparathyroidectomized-hypophysectomized rats:				
(a) Thyroxin	Normal	Decreased	Decreased	Inactive
(b) Both thyroxin and anterior pituitary extract containing the growth hormone	Normal	Increased	Increased	Active

ossification could be expressed histologically in the cartilage in three different ways: (1) an increase in the number of cells, (2) an increase in the size of the cells and (3) an increase in the amount of intercellular matrix. In addition to changes in the cartilage, growth during a chronic experiment could be influenced also by subsequent replacement of the cartilage by bone, the rate of which might exceed that of cartilage growth, remain proportionately the same or become reduced. Since normally in the rat throughout the greater part of the life span an equilibrium is maintained between cartilage and bone formation in those bones chosen for histologic study, changes in the rate of bone formation would be clearly indicated by changes in the width of the active cartilage. In the first expression of endochondral stimulation mentioned, there would be ossification of the epiphysial disk; in the second, the epiphysial disk would remain normal in width, but there would be an increase in the

amount of cancellous bone; in the third, there would be an increase in the width of the disk. With all of these various combinations in mind, we have summarized the results of this experiment in table 5.

The effects of these changes on the width of the epiphysial cartilage are graphically shown in figure 10.

From the preceding comment it is apparent that the action of thyroxin on endochondral ossification in the rat differs in several respects from that of the anterior pituitary extract containing the growth hormone. Whereas treatment with thyroxin caused no perceptible increase in the rate of endochondral ossification in normal animals, injections of the extract resulted in stimulation of both cartilage and bone formation—the equilibrium finally established favoring the former. In thyroparathyroidectomized rats, injections of thyroxin repaired the dwarfing that

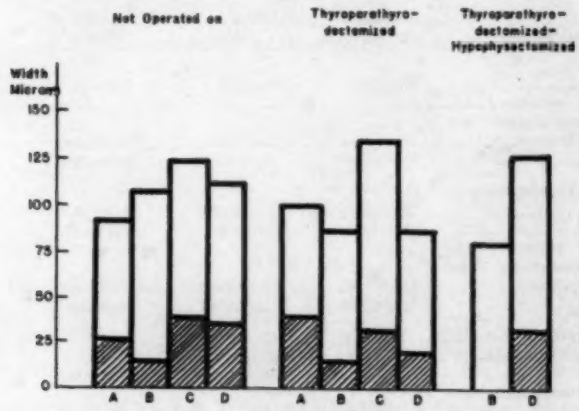

Fig. 10.—Average width of the proximal epiphysial cartilages of the tibias of female rats in series 1 (not operated on), series 2 (thyroparathyroidectomized) and series 3 (thyroparathyroidectomized and hypophysectomized). *A* indicates the control group in each series; *B*, the group given injections of thyroxin; *C*, the group given injections of an anterior pituitary extract containing the growth hormone; *D*, the group given both thyroxin and the extract.

resulted from the operation by symmetric stimulation of chondrogenesis and osteogenesis. The extract, while more effective than thyroxin in raising the body weight, gave about the same increase in body length. However, the skeletal stimulation, instead of being symmetric, favored proliferation of cartilage. In thyroparathyroidectomized-hypophysectomized rats, thyroxin produced little if any skeletal growth.

Another observation indicating that thyroxin and the extract containing the growth hormone do not influence the skeleton in the same way is that of their combined action on skeletal growth. That they act

synergistically is suggested by table 6, in which the average increase in total body length over that of the normal control is expressed in terms of percentage of the normal length.

Hammett [4] in a comprehensive study on the relation of the thyroid gland to growth in rats suggested that thyroxin acts by causing an increase in cell mass. Dott and Frasier [2] claimed that thyroxin causes early maturity and cessation of growth in dogs and cats due to a decrease in the width of the epiphysial cartilage with more active resorption along the erosion line. The Silberbergs [11] reported a marked increase in the width of the epiphysial disk and subsequent retardation of bone replacement in normal young guinea pigs fed thyroxin. The findings presented in this paper on the action of thyroxin on the skeletons of normal rats do not confirm any of the aforementioned observations. Subtoxic doses, while causing a reduction in body weight, resulted in neither stimulation nor inhibition of growth, grossly or histologically.

TABLE 6.—*Percentage Increase in Average Body Length Over Normal Control*

Group	Control	Thyroxin	Anterior Pituitary Extract Containing the Growth Hormone	Both
Not operated on	.....	— 0.7	10.2	10.7
Thyroparathyroidectomized	—23.2	— 6.1	—7.5	0.9
Thyroparathyroidectomized-hypophysectomized	.....	—21.7	....	9.0

Thyroparathyroidectomy in young animals causes a pronounced slowing of growth (rats, Hammett [4]; dogs and cats, Dott and Frasier [2]). Dott and Frasier [2] suggested that the operation causes depression of the vital activity of the cells resulting in static infantilism. However, the findings presented by the present series of animals do not resemble those in a 40 day old animal. (See Ray, Evans and Becks [16] for an illustration of the normal epiphysial disk of a 50 day old female rat.) Following thyroparathyroidectomy there are decrease in the width of the epiphysial disk until it is comparable to that of the controls at autopsy, decrease in the size and the number of cells and deposition of a layer of bone along the zone of erosion. In many respects the changes resemble those following hypophysectomy, although they are not as severe. Indeed, they may be due to changes in the pituitary gland. It is conceivable that in a chronic experiment such as this, an initial slowing of growth could occur, due to thyroparathroidectomy, and that changes in the pituitary gland could subsequently intervene, thus obscuring the picture. The possibility also arises that the growth obtained with thyroxin was due to indirect stimulation of the animal's pituitary gland since the thyro-

parathyroidectomized-hypophysectomized rat did not respond to injections. However, it must be remembered that the postoperative period before the onset of injections was considerable—long enough to allow secondary changes to take place in the bones that could prevent the response. It must also be borne in mind that the animals not operated on failed to respond to thyroxin treatment even though a latent capacity was present in these animals, as indicated by the stimulation obtained with the extract containing growth hormone. Furthermore, recent reports have shown that hypophysectomized animals will respond to injections of thyroxin (Laqueur and Freud [18]), a finding contributing still further support to the observation that these two endocrine glands can act on the skeleton independently of each other.

The type of growth obtained with the anterior pituitary extract resembles growth in a young animal in that the balance between chondrogenesis and osteogenesis favors the former to some extent. The ability of this extract to cause skeletal growth in the absence of the thyroid gland would appear to be firmly established both by the Silberbergs on guinea pigs [10] and by this laboratory on rats (present communication).

The synergistic response of the skeleton obtained with injections of both the extract containing the growth hormone and thyroxin can be explained on the basis of the histologic response. Whereas the extract gave a somewhat disproportionate growth, particularly marked in the thyroparathyroidectomized rats, combinations of the two treatments gave a more normal mature picture of endochondral ossification.

SUMMARY

Subcutaneous injections of thyroxin in normal female rats over a period of two hundred and fifty-one days caused no perceptible increase grossly or histologically in the rate of endochondral ossification in the tibia or the rib. Thyroparathyroidectomy resulted after a postoperative period of approximately three hundred and thirty days in marked dwarfing of the animals; histologically the picture of endochondral ossification at autopsy resembled that following hypophysectomy although the changes were not as severe—decrease in the size of the chondrocytes, deposition of bone along the zone of erosion, very light formation of cancellous bone and marked increase in the fat content of the marrow. Injections of thyroxin in these rats were followed by a return to the normal in gross and histologic appearance. Injections of thyroxin in thyroparathyroidectomized-hypophysectomized female rats had no effect in repairing the growth defect in these animals.

Injections of a bovine anterior pituitary extract containing the growth hormone into normal female rats for two hundred and fifty-one days

18. Laqueur, E., and Freud, J.: Acta brev. Neerland. **11**:46, 1941.

resulted in a marked increase in body weight and length. Histologically, endochondral ossification in the tibia and the rib was extremely active, the resultant picture resembling that of a young animal. In thyroparathyroidectomized rats, a similar period of injections also caused an increase in the rate of endochondral ossification. The balance between cartilage and bone formation that was finally established favored the former.

Injections of both the extract containing the growth hormone and thyroxin in normal female rats over a period of two hundred and fifty-one days resulted in a greater respone in body weight and length than that obtained with the extract alone. Histologically, endochondral ossification, although active, was more mature in type; i. e., the epiphysial disk was narrower and replacement by bone more active. This difference between the response to the extract alone and the combined hormones is more pronounced in the thyroparathyroidectomized rats. In the thyroparathyroidectomized-hypophysectomized animals, the combined extract and thyroxin treatments repaired the growth defect in contrast to the result with thyroxin alone.

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SECONDARY TUMORS OF THE HEART

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Secondary malignant tumors of the heart are not uncommon. By 1893, as reported by Hektoen,[1] the literature already contained reports of 110 cases. Since then, lengthy statistics have been compiled by Peters and Milne,[2] Symmers,[3] Morris,[4] Yater,[5] Hamilton,[6] Pollia and Gogol,[7] Scott and Garwin [8] and Lisa, Hirchhorn and Hart.[9] The frequency of occurrence varies from 0.03 to 1.4 per cent of the total number of necropsies and from 1 to 7.5 per cent of the total number of cancers. These figures closely coincide with the findings at the Jefferson Medical College Hospital, where, in a total of 4,050 necropsies, there were 640 cases of cancer, in 35 of which the heart was secondarily involved.

PATHOLOGIC OBSERVATIONS

Primary Site of Neoplasm.—Metastases in the heart have been noted from neoplasms in practically all the organs of the body. A complete list of the primary sites is given by Morris,[4] Yater [5] and Burke.[10] Table 1 shows the primary sites of the tumors in our series, the total number of cases of cancer encountered, and the number of cases in which the heart was involved. Carcinoma of the lungs involved the heart eight times in 66 cases, while carcinoma of the stomach involved the heart only once in 76 cases. This does not mean that cardiac metastasis from primary carcinoma of the lung is the most frequent, for a computation of percentages of occurrence reveals that carcinoma of the lungs, breasts,

From the Clinical Laboratories of the Jefferson Medical College Hospital.

1. Hektoen, L.: M. News, Philadelphia **63**:571, 1893.
2. Peters, H. Le B., and Milne, L. S.: New York M. J. **94**:383, 1911.
3. Symmers, D.: Am. J. M. Sc. **154**:225, 1917.
4. Morris, L. M.: Am. Heart J. **3**:219, 1927.
5. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.
6. Hamilton, R. L.: Am. J. Cancer **18**:919, 1933.
7. Pollia, J. A., and Gogol, L. J.: Am. J. Cancer **27**:329, 1936.
8. Scott, R. W., and Garwin, C. F.: Am. Heart J. **17**:430 1939.
9. Lisa, J. R.; Hirschhorn, L., and Hart, C. A.: Arch. Int. Med. **67**:91, 1941.
10. Burke, E. M.: Am. J. Cancer **20**:33, 1934.

esophagus, larynx and small intestine, respectively, occurs with about equal frequency. Lymphosarcoma, Hodgkin's disease and melanoma have in general a higher frequency rate than carcinoma.

Involvement of Other Organs.—The heart is a poor nidus for metastatic cells, owing probably to its constant activity (Yater [5]). This is substantiated practically by the fact that secondary growths of the heart are rarely seen without dissemination of the neoplasm throughout the body. In this series there was a single case in which the only metastasis was a solitary mass in the left ventricle. The patient had squamous cell carcinoma of the larynx with extension to the immediately

TABLE 1.—*Primary Sites of Tumors*

Primary Tumor	Cases	Number in Which Heart Was Involved
Carcinoma of		
Stomach	76	1
Lung	66	8
Esophagus	38	3
Rectum	34	1
Pancreas	31	1
Bladder	28	1
Primary site unknown	27	1
Breast	26	3
Larynx	17	2
Small intestines	8	1
Tongue	6	1
Sphenoid sinus	1	1
Other tumors		
Lymphosarcoma	16	3
Hodgkin's disease	13	2
Melanoma	8	2
Sarcoma (thigh)	5	1
Thymoma	5	1
Pleural endothelioma	4	1
Myosarcoma of cervix	1	1

adjacent structures. In the remaining 34 cases there was either extensive generalized metastasis or widespread pulmonary and mediastinal permeation by tumor tissue.

Cardiac Involvement.—The neoplastic growths are found in all parts of the heart. No portion is immune, and no portion is favored more than another. In our series there were 8 cases which showed myocardial involvement alone, 7 pericardial, 17 both pericardial and myocardial, 4 endocardial and 4 valvular leaflet. There were 13 cases with pericardial effusion. In 6 of these the fluid was clear, in 3 amber, in 3 bloody and in 1 fibrinous. There were 2 cases in which the pericardial cavity was completely obliterated by fibrous tissue.

Grossly, the pericardium may take on one of three appearances. It may be covered with few or numerous small or large discrete nodules resembling those in other organs. In some cases it may be covered with fibrinous material, the tumor being recognized with certainty only microscopically. Finally, a heavy deposition of fibrous tissue may entirely or partially obliterate the pericardial cavity. Here again the tumor can be identified only microscopically, for macroscopically the obliteration does not differ from any other fibrous involvement.

In the myocardium the tumor nodules vary greatly in size, from pinpoint to several centimeters in diameter. Some are sharply circumscribed, while others merge gradually with the surrounding muscle tissue and are ill defined. When small, the latter are often difficult to distinguish from myocardial scars. The larger ones often show areas of softening and necrosis, while the smaller ones are more frequently firm and homogeneous.

The endocardial and valvular tumors are of two types. One type is nodular and its free border is smooth and glistening. It probably originates in the subendothelial connective tissue, and as it protrudes into the cavity, it pushes before it the unbroken endothelial lining. The other type is a direct implantation on the endocardium from the blood stream. Being rough, the original tumor implant is sometimes covered with blood elements and then has the appearance of an ordinary vegetation. At other times the coagulation of blood is so marked that a large thrombus is formed and completely camouflages the original implant.

As to the side of predilection in the heart, findings by different observers vary. Yater [5] found the right side involved more frequently than the left. In the series reported by Scott and Garwin [6] the left side was more often involved. Morris [4] stated that in his cases the distribution was evenly divided. Our observations fall in the latter category, the neoplastic process being found just as frequently on the left as on the right side.

Mechanism of Cardiac Involvement.—The tumor reaches the heart by any of three routes. One of the most common is by direct extension from the lungs or the mediastinal structures. In this series 14 cases fell into this group.

The second route, about equally important, is by way of the blood stream. Invasion by this route occurs either through the coronary arteries or by direct implantation of the tumor on the endocardial surfaces. With reasonable certainty we could include in the former group 13 of our cases, while only 1 case could be inserted unequivocally into the latter group. This was a case of myosarcoma of the cervix uteri. Necropsy disclosed direct extension of the tumor into the pelvic veins and the inferior vena cava. Attached to a leaflet of the tricuspid

valve was a large tumor thrombus. There was no other cardiac involvement. In the remaining cases in which endocardial nodules were present, it could not be ascertained whether they occurred by direct implantation or by way of the coronary arteries.

Finally, there is a lymphatic spread. This occurs either by way of the azygos veins (Burke[10]) or by a retrograde movement through the tracheobronchial lymph channels (Yater[5]; Burke[10]; Scott and Garwin[6]). In this series the mediastinal nodes were extensively involved in 5 cases, and since no direct extension of the tumors could be traced, it is assumed that the tumor cells permeated the lymphatics and reached the heart either by a retrograde process or by an involvement of the azygos veins. In none of these cases, however, was there direct evidence to substantiate this supposition.

CLINICAL FINDINGS

It is of importance not only from the academic but also from the therapeutic and prognostic standpoints that an antemortem diagnosis of secondary cancer of the heart be made. Although the chances for a cure are meager or entirely out of the question yet, as illustrated by Shelbourne and Aronson,[11] high voltage irradiation of the cardiac region may greatly ameliorate the patient's condition. In May 1930 Willius and Amberg[12] reported the first case of metastatic cancer of the heart diagnosed before death. Their patient had Ewing's sarcoma of the left femur. Two years after the diagnosis was first made, evidence of marked cardiac failure developed. Since the publication of that report there have been only scattered cases in which an antemortem diagnosis was reported made. In November 1930 Fishberg[13] reported 3 cases in which metastatic lesions of the heart were strongly suspected before death. In 1935 Shelbourne[14] described the first case of primary cancer of the heart diagnosed ante mortem. In 1937 Smith[15] added 2 cases of secondary tumor, and in 1940 Shelbourne and Aronson[11] added still another. There may have been other reports of such sporadic cases, which escaped our attention, but even so, as compared with the total number of cases described, there have been very few in which the condition was diagnosed before death.

There have been several attempts to list signs and symptoms which would facilitate antemortem recognition. Chief among them are those of Fishberg,[13] Yater[5] and Lisa, Hirschhorn and Hart.[9] Briefly stated, the signs and symptoms resolve themselves to any of those referable to

11. Shelbourne, S. A., and Aronson, H. S.: Ann. Int. Med. **14**:728, 1940.
12. Willius, F. A., and Amberg, S.: M. Clin. North America **13**:1307, 1930.
13. Fishberg, A. M.: Am. J. M. Sc. **180**:629, 1930.
14. Shelbourne, S. A.: Ann. Int. Med. **9**:340, 1935.
15. Smith, D. S.: J. A. M. A. **109**:1192, 1937.

a previously normal heart appearing without due cause in a healthy person or in one with known cancer. These include progressive cardiac failure which fails to respond to the usual treatment, dyspnea, angina pectoris, coronary disease, arrhythmia, heart block, auricular flutter and fibrillation, cardiac murmurs of any sort over any of the orifices, pericardial effusions either simple, hemorrhagic or fibrinous, and mediastinal compression. The roentgen and electrocardiographic studies and the cytologic examination of pericardial effusions may yield important results. It is obvious that great difficulty will often be encountered in ruling out the ordinary causes of such disorders, which occur in patients of the same age group.

A clinical analysis of our 35 cases is summarized in table 2.

In going over the histories of the patients, all the signs and symptoms mentioned by previous observers were carefully sought for, but, as seen

TABLE 2.—*Clinical Analysis*

Clinical Findings	Number of Times Observed
Cardiac failure	3
Dyspnea	15
Angina	2
Irregular rhythm	3
Murmurs	2
Friction rub	2
Cardiac enlargement	7
Roentgenographic evidence of change	5
Electrocardiographic evidence of change	3
Known cancer	17

from table 2, few were found. Cardiac failure occurred three times. The cases included here were those in which record was made of edema of the extremities, enlargement of the liver and fluid in the serous cavities. In the first case necropsy showed large myocardial tumor masses, and in the second, tumor vegetations on the aortic cusps. In both of these the heart failure could be ascribed to the cardiac neoplasm. In the third case, however, the tumor was only 1 cm. in diameter, and since it was situated in the left ventricular wall, it did not appear to take any part in the cardiac failure. Dyspnea was the most common symptom. In 13 of the 15 cases it was due to extensive involvement of the mediastinum by the tumor and compression of the air passages. In only 2 cases could it be ascribed to the cardiac metastasis. In both cases the nodules were multiple and there was no mediastinal involvement. Precordial pain was present in 2 cases. Arrhythmia was noted three times. One of the patients with this symptom showed only an irregular pulse, one an occasional extra systole and one irregular rhythm. Murmurs occurred in 2 cases only. Both were systolic and apical. In one case

autopsy showed tumor nodules throughout the entire myocardium; in the other, a large tumor thrombus on one of the tricuspid leaflets. Pericardial friction rub was present in 3 cases. Each of the patients at necropsy was found to have pericardial involvement. Clinical cardiac enlargement was observed in 7 cases. This checked with the roentgen findings in 3 cases. In 2 others the heart was not enlarged roentgenographically, while in 2 cases a roentgen examination of the heart was not made.

Roentgen films of the heart were taken in 25 cases. In only 5 of these did they show positive changes. In 4 of the 5 instances the description read "the heart is displaced to the right." In 3 cases necropsy showed tumor masses in the pericardial cavity on the right side; in the fourth, hemorrhagic pericarditis. In the fifth case with positive roentgen findings, there was a "nodular shadow in the left hilar region which could be a tumor mass or simply part of the cardiac silhouette." At autopsy it proved to be a tumor mass in the hilus of the left lung which had also infiltrated the left auricle.

Electrocardiographic tracings were obtained in 3 cases only. In one of these the diagnosis was coronary sclerosis. At necropsy there was found diffuse pericardial fibrosis in which were embedded tumor cells. In a second case the diagnosis was a "moderate degree of myocardial impairment." At necropsy there was found in the left ventricle a small endocardial and subendocardial nodule, measuring 6 mm. in diameter. The heart was slightly enlarged. In a third case the diagnosis was "mild myocardial damage." At necropsy the pericardium and the endocardium of the right ventricle were studded with small miliary nodules, which in the latter instance extended into the trabeculae.

Typical findings of coronary occlusion, heart block, auricular fibrillation and flutter, and clinically evident pericardial effusion were not found in any of the 35 cases encountered.

COMMENT

Although half of the patients (17 of 35) were known to have cancer elsewhere in the body early enough to have enabled an antemortem diagnosis to be made, in each instance the findings were so obscure and protean that in none of the cases was the condition of the heart diagnosed before death. There is no single symptom or sign, or group of symptoms or signs, which enables a clinical diagnosis of cardiac metastasis to be made. One must be always mindful of the possibility of metastatic cancer in a patient who has any unaccountable symptoms or signs referable to the heart. This is especially true in those cases in which these abnormalities develop subsequent to the recognition of cancer elsewhere in the body. Aids, such as roentgenograms, electrocardiograms and

cytologic studies of pericardial fluid when available are indispensable. In most instances, by the time the tumors have metastasized to the heart, the general involvement is already widespread and a chance of cure is out of the question. An early diagnosis, however, serves two purposes. First, it is of value in prognosis and, second, the life of the patient may be prolonged and his comfort promoted if high voltage radiation is directed toward the heart.

SUMMARY

Thirty-five cases of secondary cancer of the heart are presented. The salient pathologic and clinical features are described, and an attempt at correlation of the two is made. In none of the cases in this series was the cardiac metastasis diagnosed before death.

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FIBRINOID NECROSIS IN ARTERIOSCLEROSIS

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NEW YORK

The term "fibrinoid degeneration," introduced first by Neumann,[1] in 1880, designates the presence within connective tissue of a homogeneous refractile substance exhibiting the tinctorial behavior of fibrin. It has been observed in a variety of locations and in association with most different morbid processes. Opinions as to the exact nature of the substance have differed.

Königer,[2] in 1903, recognized fibrinoid masses in his studies on endocarditis. He noted that the collagenous fibrils of the adjacent healthy connective tissue fused into the homogeneous masses as if fibrin were embedded on the connective tissue strands. It was his opinion that the fibrinoid appearance was associated with an essential alteration of collagen and that the substance in question was not actual fibrin.

Askanazy,[3] in 1907, described a definite fibrinoid zone in the base of the gastric peptic ulcer, composed microscopically of uniformly smooth, intensely eosinophilic, glairy clumps or bands devoid of cells or nuclei and merging gradually into the surrounding healthy connective tissue. The paucity of the elements of organization and the frequent occurrence of mature vessels and nerves traversing the fibrinoid zone lent weight to the view that the lesion represented necrotic connective tissue.

On the other hand, Mallory,[4] in 1912, expressed the belief that the mural fibrin-like plaques in syphilitic aortas in which compression of the vasa vasorum by gumma had led to the formation of ischemic necrotic areas were due to deposition of varying amounts of fibrin and subsequent stimulation of fibrous tissue proliferation.

Special emphasis was attached to the question of fibrinoid substance in Leary's [5] study of the morphogenesis of coronary occlusion. In 6 of

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1. Neumann, E.: Arch. f. mikr. Anat. **18**:130, 1880; Virchows Arch. f. path. Anat. **144**:201, 1896.

2. Königer, M.: Arb. a. d. Path. Inst. zu Leipzig **1**:2, 1903.

3. Askanazy, M.: Therap. Monatsh. **21**:443, 1907; Virchows Arch. f. path. Anat. **234**:111, 1921.

4. Mallory, F. B., in Harvey Lectures, 1912-1913, Philadelphia, J. B. Lippincott Company, 1913, p. 150.

5. Leary, T.: Arch. Path. **17**:453, 1934.

the hearts of his series, a fibrinoid substance within the intima of the occluded vessel was designated as the precipitating factor in parietal thrombosis and was determined to be necrotic collagen. Leary defined the fibrinoid substance as the structureless mass occurring in an atheromatous abscess between the acicular fatty clefts. Horn [6] also expressed the belief that the fibrin-like substance of the subendothelial stroma led to parietal thrombosis within the coronary arteries. His interpretation that the fibrinoid substance represented a conglutinated mass of erythrocytes devoid of iron-containing pigment was based on the occasional finding of ghost cell outlines of erythrocytes within the mass. This coincided with a report of Krebs,[7] in 1889, in which the hyaline appearance of a contracted, several day old thrombus was regarded as due to compressed erythrocytes despite the absence of iron-containing pigment.

Jucker [8] recently investigated the fibrin-like masses in the intima of arteriosclerotic vessels and noted their presence deep within hyperplastic plaques distant from both lumen and mural blood supply. He regarded the lesion as an irreversibly altered state of connective tissue but was puzzled by the complete loss of its fibrillar appearance. He suggested, as Askanazy [8] had previously, that loosening of connective tissue due to damage of the cement substance allowed seepage inward of fibrinogen either to engulf the individual fibers completely or to pool within the ground substance and form complex colloidal products.

Jaeger [9] described a fibrinoid appearance of the intima of peripheral vessels in thromboangiitis obliterans and periarteritis nodosa as well as in arteriosclerotic vessels. In this work he emphasized the importance of silver preparations for the microscopic analysis. He demonstrated that in the layered portion of a thrombus where a yellow-brown background was assumed in the course of the silver impregnation, argyrophilic fibers were absent. However, beneath the thrombus the mural fibrinoid mass did contain silver fibers, a constant property which established a close relationship between fibrinoid degeneration and mature connective tissue.

Strongly opposed to the opinion that fibrinoid is other than an infiltration of true serum fibrin was the report of Clark, Graef and Chasis.[10] Limiting their investigation to arteriosclerotic and syphilitic aortas and arteriosclerotic coronary vessels, they found no support for

6. Horn, H., and Finkelstein, L.: Am. Heart J. **19**:655, 1940.

7. Krebs, W.: Hyaline Thrombosen in embolischen Lungeninfarkten, Tubingen, H. Laupp, Jr., 1887.

8. Jucker, P.: Virchows Arch. f. path. Anat. **295**:301, 1935.

9. Jaeger, E.: Virchows Arch. f. path. Anat. **289**:526, 584, 1932.

10. Clark, E.; Graef, I., and Chasis, H.: Arch. Path. **22**:183, 1936.

the belief that damaged, necrotic collagen could assume the tinctorial qualities of fibrin.

From the foregoing discussion it becomes evident that the question of the true nature of the so-called fibrinoid material in arteriosclerosis and its differentiation from fibrin has not yet been satisfactorily answered. In order to establish such a differentiation, I have added the biochemical attack of controlled tryptic digestion. In 1880 Ewald and Kuhne [11] reported extensive studies on tissues subjected to the lytic action of trypsin. The activity of the pancreatic ferment was found to vary in proportion to the potency and the age of the specific product, the duration of its use and the species of animal from which the trypsin was extracted. Mall [12] recorded that the activity of the trypsin could be carefully controlled by the degree of alkalinity. Keratin, fat and carbohydrate are resistant to the proteolytic powers of trypsin. The simpler proteins, such as mucin, chondromucin, elastin, fibrin, albumin and globulin, are readily broken down. The parenchyma of the internal organs and all types of muscle tissue are rapidly digested. Elastic fibers swiftly disappear on exposure to the ferment. Collagen is the most resistant of all protein structures.

The method of this study, therefore, was to expose the fibrin-like masses within arteriosclerotic aortas and peripheral vessels of the lower extremity to the proteolytic action of trypsin in a well regulated and controlled fashion.

MATERIAL AND METHODS

Blocks were cut from patent and thrombosed arteriosclerotic aortas, syphilitic aortas with secondary atherosclerosis and peripheral arteriosclerotic arteries. For a study of the earliest changes, sections were made from the patent peripheral vessels and from intact or ulcerated nonthrombotic aortas. The more complex states were analyzed from the occluded vessels. No attempt was made to identify the fibrinoid change grossly. When the fibrin-like masses were noted in random sections taken from the patent vessels, the blocks represented were designated for further study. In the occluded vessels, the sections were always made through what appeared to be the initial site of the thrombus formation.

The material was routinely fixed in 8 per cent solution of formaldehyde. Embedding in paraffin followed as a rule. Microscopic preparations were made by Mayer's hematoxylin-eosin method, the Weigert elastic-Van Gieson method, the Van Gieson method and the Mallory aniline blue method as modified by Heidenhain, for connective tissue. The silver impregnation of Bielschowsky as modified by Otani was employed routinely.

The tryptic solution was prepared from fresh dry trypsin of standardized proteolytic potency. A saturated aqueous solution was made by adding as much of the dry powdered ferment as possible to any desired quantity of distilled water.

11. Ewald, H., and Kuhne, W.: Verhandl. d. naturhist-med. Ver. zu Heidelberg **1**:451, 1880.

12. Mall, F. P.: Johns Hopkins Hosp. Rep. **1**:171, 1896.

Vigorous shaking was necessary to dissolve the maximum amount of trypsin. A p_H range between 7.0 and 9.0 was carefully established by slowly adding sodium carbonate. Alkacid paper was used to determine the end point. A fresh solution of the trypsin was prepared each day immediately before it was to be used.

The microscopic section to be prepared for trypsin digestion was deparaffinized in the routine manner, washed in water and covered with two pieces of filter paper of somewhat larger dimensions than the glass slide bearing the predigested specimen. A second glass slide was placed over the filter papers. A no. 18 rubber band was then loosely wrapped in double fashion about the width of the two slides. The double slides were then placed flatly in the trypsin solution in batches of six to eight. These precautions were necessary in order to prevent the section from washing off the slide or folding on itself. Careful attention to the size of the rubber band was warranted, so that no force was exerted in any one place; this would have disturbed the even digestion of the entire section. A small amount of either chloroform or toluene was added to prevent bacterial growth, and the solution was placed in an incubator at 37 C.

On each individual glass slide bearing a microscopic section to be subjected to trypsin digestion, there was placed a control of some true fibrin-containing material: A glass bead thrombus, fibrinous lobar pneumonia tissue, a splenic artery embolus or uremic fibrinous pericarditis tissue was selected for this purpose. In each control large masses of fibrin were present. All of the fibrinous masses were comparable in their susceptibility to the digestive action of trypsin. Most of the subsequent work was carried out with the pericardial sections used for the control because of the presence of myocardium in the section. Heart muscle was found to lose its identity as rapidly as true fibrin disappeared. Thus a doubly checked control was available by the use of sections of uremic fibrinous pericarditis with underlying myocardium.

The digestion of the fibrin was found to vary so widely with each day's preparation that it was necessary to incubate batches of about eight serially obtained sections from one block in 500 cc. of the trypsin solution at one time. With the best available ferment, the fibrin control would be digested in about one and three-fourths to three hours. The sections were withdrawn from the solution after one and one-half hours' immersion and every fifteen minutes thereafter. On removal from the solution, the double slide preparation was dehydrated in the alcohols and in acetone. The top slide was removed without disturbing the filter papers. The section was thoroughly dried before the filter papers were removed in order to obtain flat unwrinkled preparations. Routinely the material was subjected to the Van Gieson connective tissue stain, selected because of the simplicity of its application. All other technics described for the predigested material were used whenever indicated.

MICROSCOPIC APPEARANCE OF THE FIBRINOID SUBSTANCE IN ARTERIES

The fibrinoid substance occurs as a homogeneous, structureless refractile mass beneath the formed elements of the thrombus in occluded arteriosclerotic vessels or within the intima itself of patent arteriosclerotic vessels. Its presence in syphilitic aortas beneath bland thrombi is associated with the secondary intimal atherosclerosis. The fibrinoid change is not pathognomonic for any specific disease process.

Tinctorially, the fibrinoid substance is characterized by its close similarity to fibrin. Each has an intense affinity for eosin; each gives a bright canary yellow reaction in the Van Gieson stain, a brilliant red one in the Heidenhain modification of the Mallory connective tissue preparation and a blue-black response in Weigert's fibrin stain and in the phosphotungstic acid–hematoxylin preparation. Contrary to the observations of Clark, Graef and Chasis,[10] optical differences exist between fibrin and fibrinoid matter. High magnification reveals a fibrillated appearance of the former, which is conspicuously lacking in the latter. Welch,[13] in 1920, noted a similar closely matted and granular property of fibrin situated around the initial platelet deposit in thrombus formation. In contrast, a fibrinoid mass presents a smooth, homogeneous, smudged appearance.

The location of the fibrinoid mass in patent vessels permits speculation as to its morphogenesis. Repeatedly, islands of the refractile smooth structureless substance are found deep within intimal plaques entirely surrounded by healthy connective tissue. The view that fibrinoid is merely fibrin pressed into the intima from the lumen (Clark, Graef and Chasis [10]; Mallory [4]) is difficult to reconcile with this finding.

Although presenting evidence that fibrinoid represents an altered state of collagen, Jucker [8] was unable to explain satisfactorily how the fibrillary structure of mature connective tissue could entirely disappear in the smooth mass that is characteristic of fibrinoid. However, the application of silver impregnation with demonstration of argyrophilic fibers therein reveals that an altered fibrillary framework is retained. Characteristically, a sparse network of interlacing, occasionally fragmented, rather thick black silver fibers course through the fibrinoid mass. They are absent in fibrin. In the fibrinoid myocardial connective tissue septums of acute rheumatic fever, Klinge [14] demonstrated conspicuous argyrophilic fibers, not present in normal collagen tissue, which assumes a brown shade in silver impregnation. He expressed the belief that the difference is due to the fact that the interfibrillary ground substance swells in rheumatic fever and that the normally tightly compressed fibrils become separated and therefore are more easily accessible to silver impregnation. A similar opinion has been expressed by Mallory and Parker [15] to account for the different silver staining of reticulum fibers and mature collagen bundles. The fragmented thick black argyrophilic strands within the fibrinoid masses in arteriosclerosis may be better

13. Welch, W. H.: Papers and Addresses, Baltimore, Johns Hopkins Press, 1920, vol. 1, p. 110.

14. Klinge, F.: Ergebn. d. allg. Path. u. path. Anat. **27**:1, 1933.

15. Mallory, F. B., and Parker, F.: Am. J. Path. **3**:515, 1927.

interpreted, however, as relatively healthy intact fibers resistant to the injuring factors which have largely destroyed the bulk of the collagen fibers.

Examination of the border of the fibrinoid mass reveals that the healthy connective tissue undergoes a gradual change on merging into the structureless substance. The fibrous bundles split and fray into individual fibers and thus extend onward into the fibrin-like plaques, where they can be well demonstrated by silver impregnation. The concept of progressive connective tissue degeneration in the evolution of the atherosclerotic plaque is supported by the histologic picture found in the necrotic "atheromatous abscess" within the intima of vessels affected with advanced arteriosclerosis. The connective tissue bundles of the capsule of the necrotic atheroma separate into fibrillary components; the individual fibers fray and dangle freely in the necrotic fatty plaque. From this it would appear that the atheroma has reached a far advanced stage of necrosis with complete dissolution of its stroma, while the fibrinoid mass represents less drastic alteration, retaining a reduced fibrillary structure discernible in silver preparations.

EFFECT OF TRYPTIC DIGESTION

Fibrinoid Masses Within Arteriosclerotic Peripheral Vessels.—Fibrinoid necrosis was initially investigated in the peripheral arteries, where the lesion was localized to hyperplastic intimal plaques. In this location, the mass was considered to be present in its simplest form. The examination embraced patent and occluded vessels exhibiting on the luminal surface fibrin-like superficial bands fusing intimately with the subendothelial stroma, a fibrin-simulating layer beneath fresh bland parietal thrombi and/or clumps and sheetlike masses deep in the intima. Older, completely canalized thrombi were omitted. A careful differentiation was made in all instances between the appearance of fibrinoid masses and the necrotic stroma of intimal atheromas, although both were frequently found fusing into each other.

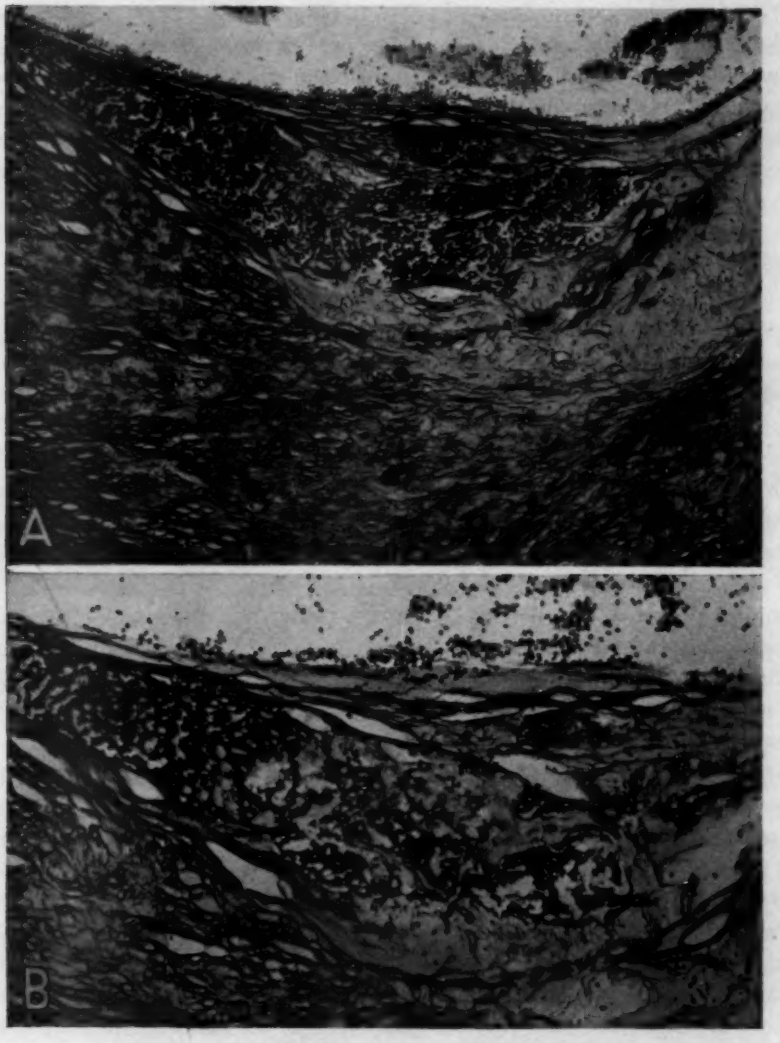
Tryptic activity materially altered the microscopic anatomy of the vessel exposed for a period sufficient to digest all available fibrin from the control section. The smooth muscle of the media was digested, leaving a resistant pale smudgy pink (Van Gieson connective tissue stain) structureless mass in which the collagen framework was clearly defined. The nuclei were promptly digested. The white blood cells entirely disappeared to leave a nondescript residue. Erythrocytes were more resistant and remained intact. Fibrin, serum proteins and edema fluid were entirely displaced by an ill defined amorphous material apparently composed of undigested carbohydrates and fats. The location of an occluding luminal thrombus was indicated only by the remaining red blood cells. Endothelial cells were digested. In a parallel digestion of

the chicken fat clot, blood platelets were found to disappear as rapidly as fibrin. The colloidal gel ground substance of connective tissue investigated in the umbilical cord rather than in the arterial wall, where its presence is inferred rather than seen, was more resistant than fibrin. The young, immature fibers of connective tissue were consumed before the mature, collagenized bundles but were still intact when fibrin had already been digested. Elastic fibers disappeared before even the most immature collagen fibrils but were frequently found after all fibrin had been consumed. Collagen fibers remained unaltered as the framework of the arterial wall when the proteolytic ferment had digested all else. In the most advanced states of digestion, even the collagen bundles of fibrous tissue were eventually proteolyzed to leave a completely amorphous nondescript mass.

The islands of fibrinoid substance located deep in the subsurface portion of the intima remained intact and unaltered by the tryptic ferment at that point when complete digestion of fibrin had taken place. Whereas in the control section of a site of fibrinous pericarditis nothing remained to indicate the layer of fibrin other than scattered erythrocytes, the fibrinoid in the intimal plaque remained homogeneous, intensely eosinophilic and refractile. A fibrillary pattern was no more evident than before tryptic digestion. By means of the special connective tissue stains, the mass was found to have retained all the tinctorial properties of the predigested fibrinoid (figure). At the edge of the lesion, the bundles of healthy bordering connective tissue separated into individual fibers and merged in a gradual fashion with the fibrin-simulating mass. The silver preparation revealed both fine and thick interlacing argyrophilic fibers coursing through the fibrinoid substance and fusing into the fibrillar components of the bordering fibrous tissue.

In the peripheral artery in which a fibrinoid mass was found beneath the layered elements of a thrombus, fusing as a homogeneous layer into the subendothelial connective tissue, the tryptic digestion, controlled to the point of complete consumption of fibrin, produced a more complex picture. The thrombus always vanished completely; at its base, the fibrinoid layer remained in varying amounts. If the mass was so extremely necrotic that the silver preparation demonstrated no argyrophilic fibers but rather a smudged solid appearance, much of the lesion would be digested; only in close proximity to the adjacent healthy connective tissue did irregular clumps or narrow uneven bands remain. When the fibrinoid appearance evidenced a less severe damage of connective tissue (i. e., argyrophilic fibers were demonstrated within the lesion by the silver technic) much greater residue would be found after exposure to the ferment. Occasionally a mass of trypsin-resistant fibrinoid could be depicted out in the luminal thrombus itself, the presence of which in the section prior to digestion was unsuspected and which

was demonstrated solely by the tryptic digestion. Such chunks of undigested material represent the fragmented ragged walls of an irregular undermined atheromatous ulcer.



A, fibrinoid necrosis of an arteriosclerotic plaque (Mallory azan preparation; × 93); *B*, same plaque after controlled tryptic digestion (Mallory azan preparation; × 135).

Fibrinoid Masses in Atherosclerotic and Syphilitic Aortas.—The atheromatous-thrombotic lesion within the aorta in cases of extensive arteriosclerosis associated or not with syphilis presented after tryptic

digestion by far the most complex picture for interpretation. The thrombus prior to digestion usually presented a homogeneous bland hyaline appearance, indicative of the long duration of its deposition. In the hematoxylin and eosin preparation the luminal mass appeared to fuse solidly into the extensive underlying ulcerated atheroma. It was impossible by tinctorial technic to establish definitely the border of the fatty plaque, the presence of true fibrinoid degeneration of collagen over the atheroma and the transition between fibrinoid and overlying hyaline thrombus. Tryptic digestion, carried to the point of complete consumption of fibrin, revealed that the thrombus would rapidly be digested, that resistant fibrinoid remained if present but that the necrotic stroma within the atheroma would largely have disappeared. Within the mass of the true thrombus, trypsin-resistant clumps of fibrinoid would frequently be found, demonstrating either that the thrombus was laid down on a very irregular ulcerated surface or that actual chunks of the necrotic collagen were knocked off by the eddying blood stream at the time of the thrombus formation. Trypsin-resistant fibrinoid masses were not satisfactorily uncovered in all blocks from sites of aortic thrombosis. Only in the specimens in which argyrophilic fibers were demonstrated beneath the thrombus formation was fibrinoid successfully delineated by the digestive process. If the collagen was so severely injured that all argyrophilic fibrillar content was lost, no selective proteolytic differentiation was manifest on the use of the tryptic solution.

COMMENT

Fibrinoid masses may occur as superficial clumps or bands associated with bland parietal thrombi, or as deep sheetlike masses within the substance of intimal plaques of arteriosclerotic vessels. On microscopic inspection of these masses it has been noted that the substance appears homogeneous, acellular, solid and refractile; closer inspection reveals a smudgy glare contrasting with the closely matted fibrillated appearance of true fibrin. Silver impregnation reveals an irregular network of argyrophilic thick fibers, which are completely absent in fibrin deposits. Clark, Graef and Chasis [10] expressed the belief that these fibrils were either laid down by fibroblasts in the process of organization or were due to the splitting of the collagenous fiber into its component fibrils (Mallory and Parker [15]). Unless a luminal thrombus overlying the fibrinoid mass had stimulated organization and cellular response, leukocytes, capillary budding and fibroblastic proliferation were usually absent in the material used in this study. The association of the fibrinoid masses and the elements of organizing inflammation was rare. It is therefore not likely that the fibrinoid substance represents organizing fibrin (Clark Graef and Chasis).

The foregoing conclusions, however, at best represent an analysis of microscopic observations open to different interpretations. Controlled tryptic digestion definitely differentiates the relatively resistant fibrinoid substance from the readily consumed fibrin and makes untenable any concept that the fibrinoid substance represents a phase in the evolution of deposited or imbibed blood elements in the walls of arteriosclerotic vessels. Whereas the results will not permit absolute identification of fibrinoid, a problem ultimately left to the chemist, the resemblance in its protein structure to mature connective tissue in that both resist tryptic digestion will support the contention that fibrinoid is altered collagen.

The view expressed by Jucker [8] and Askanazy [3] that the fibrinoid change is the result of seepage of fibrinogen between the fibrillar components of connective tissue to form clumps on the altered fibrils is unsupported by the findings within the fibrinoid mass subsequent to tryptic digestion. No reappearance of a fibrillary structure is manifest in the specimens subsequent to exposure to the proteolytic ferment, and the characteristic thick silver fibers of the lesion remain unaltered. Their presence supports the contention that the fibrinoid change of the arteriosclerotic plaque represents incomplete necrosis of its collagen in which a few scattered relatively healthy argyrophile fibers remain intact.

SUMMARY

Homogeneous masses exhibiting the tinctorial behavior of fibrin and located within and on the arteriosclerotic plaques of the aorta and the peripheral vessels were analyzed in an attempt to establish their morphogenesis.

Fibrinoid substance could be clearly differentiated from fibrin by controlled tryptic digestion.

Evidence is submitted to establish the fibrinoid substance in arteriosclerotic vessels as partially necrotic collagen.

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SKELETAL ABNORMALITIES INDUCED IN RATS BY MATERNAL NUTRITIONAL DEFICIENCY

HISTOLOGIC STUDIES

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In recent years a number of publications have appeared which indicate that congenital malformations in the offspring of animals may be due to a faulty diet of their mothers.

Hale [1] reported that sows fed on rations deficient in vitamin A gave birth to pigs that were born blind, many without eyeballs. In addition, misplaced kidneys, cleft palate, harelip and other abnormalities were found. Moore, Huffman and Duncan [2] noticed blindness due to bone overgrowth in the optic foramen in calves born of vitamin A–deficient cows. Andersen [3] observed a high incidence of congenital diaphragmatic hernia in the young of rats bred on a diet deficient in vitamin A. Byerly and co-workers [4] reported micromelia in chicken embryos caused by a nutritional deficiency of the laying hens.

Warkany and Nelson [5] described congenital malformations in about one third of the offspring of female rats reared and bred on a deficient diet (diet 1). This diet was the Steenbock-Black rachitogenic diet no. 2965 [6] supplemented by viosterol to forestall the development of rickets. On external inspection, the defective offspring could often be recognized by shortening of the mandible and protrusion of the tongue, shortening and distortion of the extremities and various forms of syndactylism of

From the Children's Hospital Research Foundation and the Department of Pediatrics of the University of Cincinnati College of Medicine.

1. Hale, F.: Am. J. Ophth. **18**:1087, 1935.

2. Moore, L. A.; Huffman, C. F., and Duncan, C. W.: J. Nutrition **9**:533, 1935.

3. Andersen, D. H., read at the thirteenth annual meeting of the Society for Pediatric Research, Atlantic City, N. J., May 5, 1941.

4. Byerly, T. C.; Titus, H. W.; Ellis, N. R., and Landauer, W.: Proc. Soc. Exper. Biol. & Med. **32**:1542, 1935.

5. Warkany, J., and Nelson, R. C.: (*a*) Science **92**:383, 1940; (*b*) Anat. Rec. **79**:83, 1941.

6. Steenbock, H., and Black, A.: J. Biol. Chem. **64**:263, 1925.

the fingers and toes (fig. 1 *A*). In rare cases gross congenital defects of the soft tissues have also been observed. The skeletal abnormalities can be well demonstrated in specimens cleared by the Schultze-Dawson method (fig. 1 *B*). These specimens showed that certain bones were often abnormal and that other bones apparently were never affected.

Shortness of the tibia, shortness of the corpus of the mandible and fusion of certain ribs were frequently observed, while other bones, e. g., the scapula, the clavicle and the femur, were only rarely abnormal.

Thus a definite pattern of skeletal abnormalities was recognized in the offspring of the females reared and bred on the deficient diet. The

Fig. 1.—*A*, external appearance of a deformed newborn rat. Note brachygnathia, protruding tongue, shortness of arms and syndactylism of the third and fourth fingers. *B*, cleared specimen of a deformed newborn rat showing brachygnathia, shortness of ulna, absence of radius, fusion of ribs, absence of tibia and fibula.

anatomic details of the abnormal young have been described in one of our previous publications.[5b] In the present paper we wish to report the results of histologic studies of the bones which have yielded additional information concerning the nature of these developmental abnormalities.

The series of sections described in the following paragraphs have been selected as representative of the anomalies. The accompanying table indicates that the tibia was most often found affected in the abnormal offspring. In the cleared specimens different degrees of shortening and absence of the ossified part of the tibia were observed.

The microscopic appearance of a series of abnormal tibias will now be described and compared with that of the normal tibia of the newborn rat.

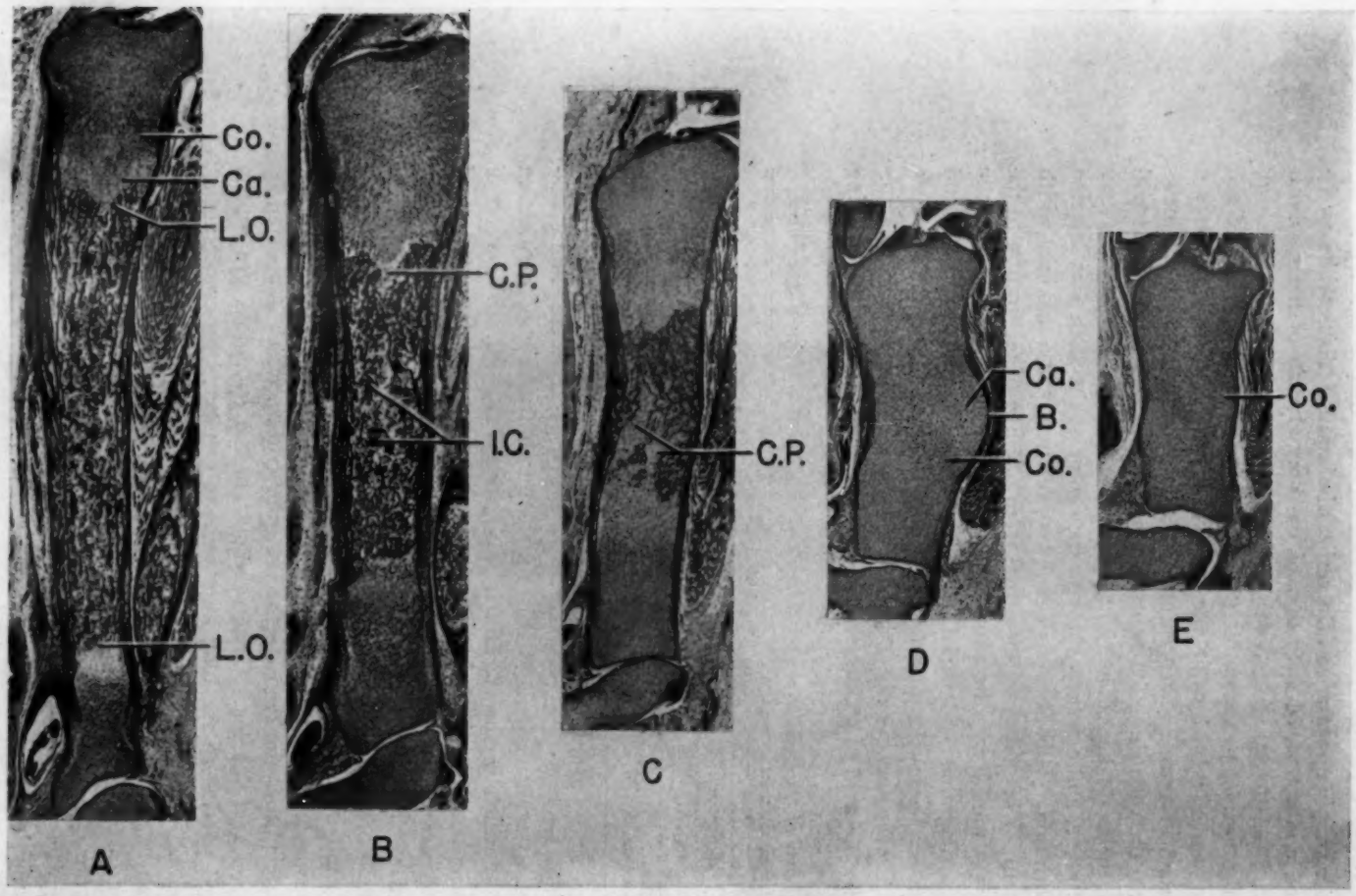
In the normal newborn rat the ossification of the diaphysis of the tibia is well advanced while the epiphysial centers of ossification are still absent. Figure 2 *A* represents a section of a normal tibia of a newborn rat, the offspring of a control female which had been reared and bred on the stock diet. The right leg was sectioned in an approximately sagittal plane, and a section through the center of the shaft was selected for description. This tibia has a length of 6.5 mm. and a maximal width of 0.8 mm. The diaphysis, measured from one line of ossification to the other, is 4 mm. long; the proximal cartilaginous end is 1.4 mm.; the distal cartilaginous end is 1.1 mm. The proximal cartilaginous end shows a few layers of joint cartilage bordering the cavity of the knee joint. Proceeding distally, one sees the typical growing cartilage followed by the area of columnar cartilage (*Co.*). The zone of calcifying

Frequency of Osseous Defects in One Hundred Cleared Abnormal Specimens

Tibia	93	Ulna	50
Mandible	80	Humerus	34
Rib	75	Hindfoot	31
Fibula	63	Maxilla	8
Radius	58	Scapula	6
Hand	54	Clavicle	6
Sternum	52	Femur	1

cartilage (*Ca.*) consists of rows of enlarged lacunas containing disintegrating cartilage cells. This zone borders the line of ossification (*L. O.*), which in this section is not entirely straight. The zone of ossification consists of the capillaries which penetrate the columns of enlarged lacunas, of osteogenetic tissue and of the newly formed bone trabeculae. The diaphysis is covered by the periosteum, consisting of an outer fibrous layer and an inner layer of osteoblasts. The cortex of the shaft is formed by several osseous lamellas which are connected by osseous bars. The anterior cortex, which lies immediately under the skin, appears thicker than the posterior cortex, which is covered by the muscles of the leg. The trabeculae of the spongiosa are mostly arranged parallel to the long axis and are connected by anastomosing trabeculae. Toward the middle of the shaft larger polygonal islands of bone can be noted. Between the osseous trabeculae well developed bone marrow can be seen.

Figure 2 *B* represents a slightly abnormal tibia. Its total length of 6.1 mm. is somewhat less than that of the control animal. The diaphysis, 2.7 mm. in length, is definitely shorter, and the end cartilages, measuring 1.8 mm. and 1.6 mm. respectively, are longer, than the corresponding



EXPLANATION OF FIGURE 2

Sections of tibias of newborn rats (× 16): *A*, normal tibia; *B-E*, tibias of different degrees of abnormality.

ABBREVIATIONS USED IN FIGURES 2 TO 4

B.—posterior bulge
Ca.—calcifying cartilage
Co.—columnar cartilage
C.P.—cartilaginous process
I.C.—islands of cartilage
I.Sp.—interosseous space
L.O.—line of ossification
M.—bone marrow
O.S.—ossified shell
O.T.—osteogenetic tissue
P.—periosteum

Co.—columnar cartilage
C.P.—cartilaginous process
L.O.—line of ossification
M.—bone marrow
P.—periosteum

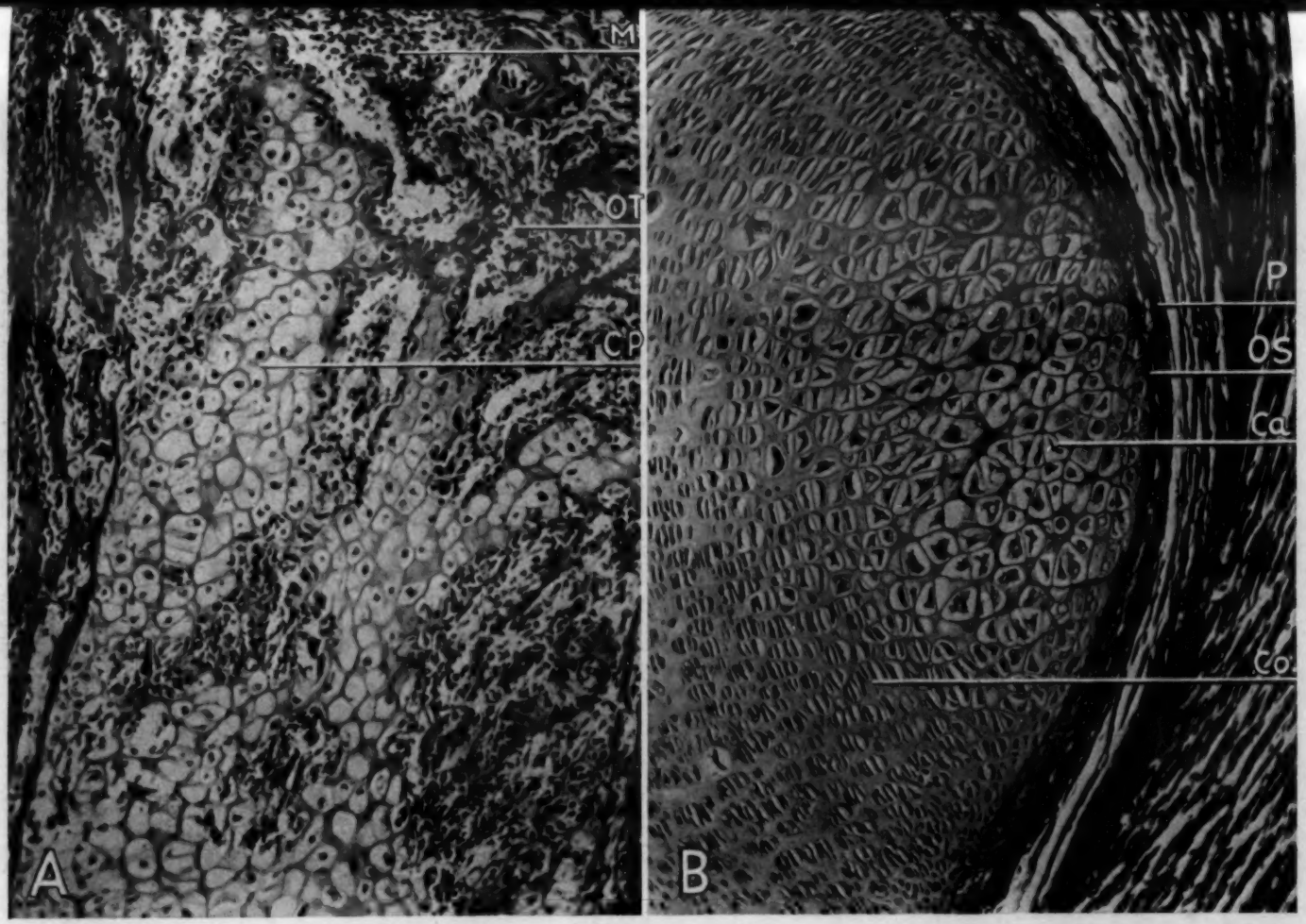


Fig. 3.—*A*, central part of the diaphysis of the tibia reproduced in figure 2 *C* (× 116). *B*, posterior bulge of the tibia reproduced in figure 2 *D* (× 116).

parts of the control tibia. Thus the cartilaginous ends are enlarged at the expense of the osseous diaphysis. The maximal width of this tibia is 0.9 mm. A tongue of cartilage (*C. P.*) projects from the proximal cartilaginous end into the diaphysis, and accordingly the line of ossification assumes a V shape. The periosteum and the cortex appear normal. The longitudinal trabeculae are well developed. In the central posterior part of the shaft several transverse bone trabeculae can be seen, and anterior to these two islands of cartilage (*I. C.*) in the stage of ossification can be discerned. Bone marrow spaces of normal appearance are visible among the trabeculae. The distal line of ossification and the distal cartilaginous end appear relatively normal.

Figures 2 *C* and 3 *A* illustrate a further degree of abnormality of the tibia. Accurate measurements of the constituents here are not possible, since there is no well defined line of ossification between the diaphysis and the distal cartilaginous end. The total length of this tibia is 4.4 mm., and the proximal cartilaginous end is 1.5 mm. long. The diaphysis has an approximate length of 1.5 mm. when measured along the posterior ossified part, and the distal cartilaginous end has a length of 1.4 mm. These measurements do not take into consideration the large cartilaginous process (*C. P.*) which extends from the distal cartilaginous end into the anterior part of the diaphysis. This tibia is 2.1 mm. shorter than that of the control, and the shortening in this case is again due entirely to the shortening of the diaphysis. The proximal cartilaginous end and the proximal line of ossification appear normal. The cortical shell of the tibia with its normal periosteum is shortened but appears unchanged otherwise. The osseous trabeculae, which are seen chiefly in the posterior part of the diaphysis, instead of lying parallel to the long axis of the tibia, seem to converge to a point in the center of the posterior aspect of the bone. Anteriorly the distal part of the diaphysis contains a large area of calcifying cartilage (*C. P.*, fig. 3 *A*) and only a few bone trabeculae. A small area of bone marrow (*M.*) containing blood elements is found in the upper third of the diaphysis; the remaining intertrabecular spaces seem to be occupied chiefly by osteogenetic tissue (*O. T.*)

The tibia reproduced in figures 2 *D* and 3 *B* has a length of only 2.8 mm. and a maximal width of 1.0 mm. It is shorter and wider than the normal tibia. It also shows an abnormal shape, caused by a marked bulge on the posterior face of the shaft (*B.*). This tibia consists almost entirely of growing hyaline cartilage with the exception of a center of calcification situated in the posterior bulge described. The bulge is covered by periosteum of normal appearance, and under this is a thin shell of bone (*O. S.*). The bulge consists chiefly of enlarged lacunas of cartilage (*Ca.*). Anterior to these lies columnar cartilage (*Co.*), with the columns of cells converging toward the bulge.

The tibia in figure 2 *E* is 2.1 mm. long and 0.8 mm. wide and consists entirely of growing hyaline cartilage. The only sign of beginning calcification in this tibia is a columnar arrangement of cells of cartilage (*Co.*) found under the slight convexity seen in the middle of the posterior outline of this structure.

The tibia in figure 4 *B* is 1.4 mm. long and 0.8 mm. wide and shows no signs of calcification or ossification.

This series of tibias reveals a variety of histologic pictures, all found in abnormal newborn rats delivered at term. In members of the same litter different degrees of abnormality may be found. Thus the tibias reproduced in figures 2 *D*, 2 *E* and 4 *B* were found in litter mates.

Other long bones, though less often affected than the tibia, show an essentially similar histologic picture, although certain local differences can be noted. A typical example of the appearance of a moderately abnormal forearm is given in figure 4 *C*. The humerus seems normal in its cartilaginous and osseous parts. The radius is definitely shortened, having a length of 2.9 mm., compared with the 5.4 mm. length of a comparable section of the radius in a normal newborn rat. The ulna likewise is shortened, having a length of 4.2 mm., compared with the 6.3 mm. length of a normal control ulna. The shortening of the ulna is due entirely to the shortening of its diaphysis, as the end cartilages are of normal length. The histologic structure of the cartilaginous ends of the radius and the ulna appears normal. With the exception of the cortical shell, the diaphysis of the radius consists almost entirely of cartilage. The cortex is thicker on the ulnar side of the diaphysis. The lacunas of the cartilage are large, and their cells show signs of disintegration. The only sign of endochondral ossification is seen in the middle of the shaft on its ulnar side, where an osteogenetic center is located. The ossification of the ulna is further advanced than that of the radius, although islands and tongues of cartilage may be seen in the diaphysis. The cortex is thick on the radial side and thinner on the opposite side. The bone trabeculae in the center of the shaft are thin and scattered irregularly. Only a few small areas of bone marrow containing blood elements are present.

Figure 4 *B* represents the lower extremity of an abnormal newborn rat. It is reproduced to demonstrate the coexistence of an apparently normal femur (*F.*) with a quite abnormal tibia (*T.*). It shows not only marked disproportion in size between the femur and the tibia but also marked difference in their histologic differentiation.

Figure 4 *A* illustrates an abnormal leg and foot. Of special interest is the syndactylism exhibited by the proximal phalanges of digits 2 and 3. The common basal phalanx of these two digits shows a center of calcification but no signs of ossification. A joint has formed between

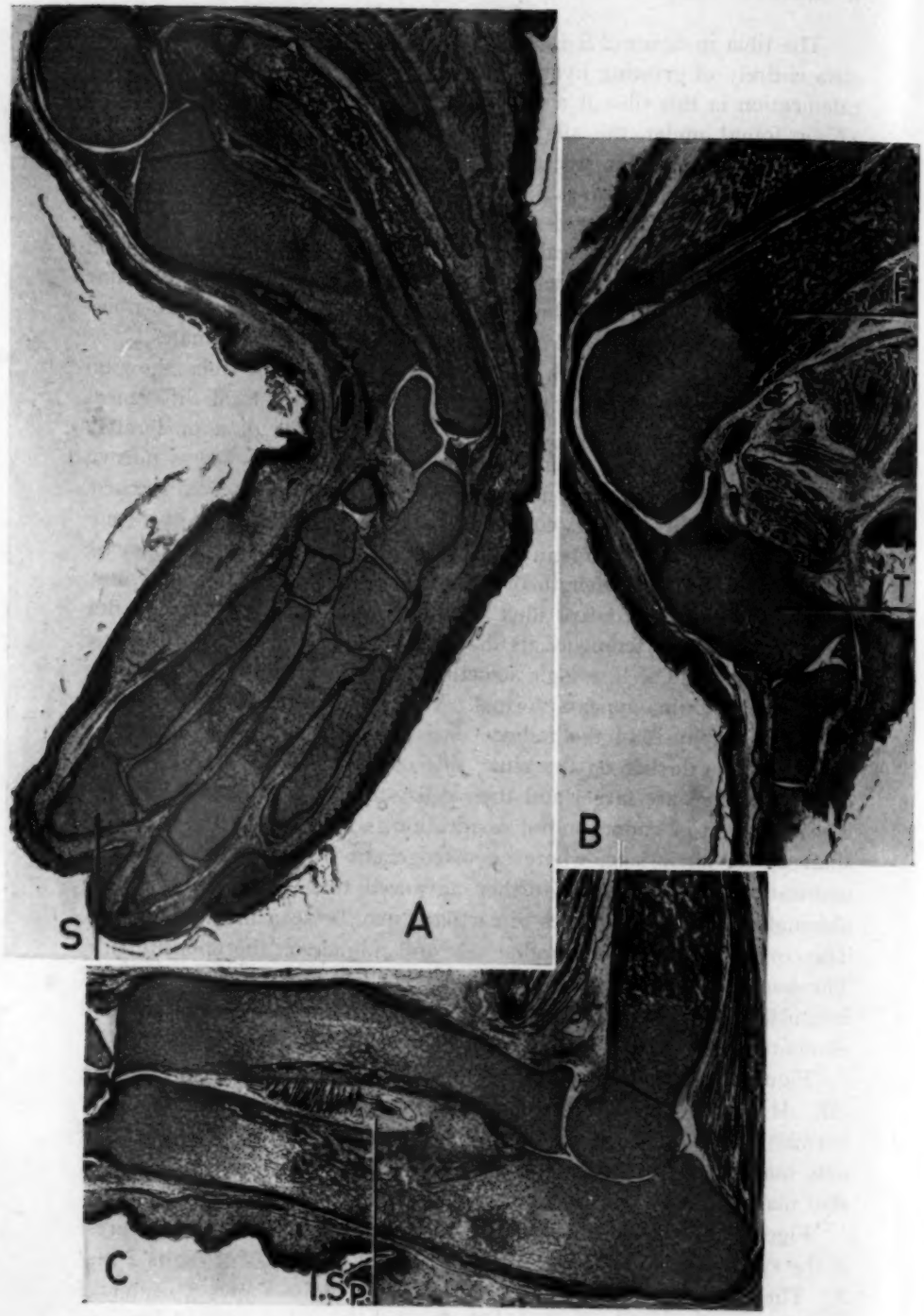


Figure 4

(See legend on opposite page)

the third metacarpal bone and this phalanx, which is, however, still in cartilaginous connection with the second metacarpal bone.

The abnormal structures described reveal a variety of histologic pictures. However, it does not seem difficult to recognize certain fundamental abnormal features which they have in common. The tibias as well as the radius and ulna illustrated in figures 2 to 4 show more or less marked delay in ossification of the diaphysis and persistence of cartilage in places where bone is found in corresponding structures of control animals of the same age. The delay is particularly noticeable in the endochondral ossification, but the structures which are more severely affected show also a lack of periosteal ossification (see tibias of figs. 2 *E* and 4 *B*). As has been mentioned, the bone trabeculae of the spongiosa show an arrangement different from those seen in the normal bone. The orderly relationship between the calcifying cartilage and the osseous parts of the bones is definitely disturbed. Cartilaginous processes and islands are found in areas of the diaphysis which should be entirely ossified. The short and straight lines of ossification seen in the normal growing long bone are supplanted by irregular and indefinite borderlines of cartilage and bone (fig. 3 *A*). In those structures in which ossification has not begun, the cartilage itself shows signs of abnormality. *D* and *E* in figure 2 show that calcification begins in the posterior part of the tibia and not in the center of the shaft as it does in the normal. This eccentric position of the centers of calcification explains the abnormal arrangement of the trabeculae seen in the later stages of development of these bones. The abnormal outline and the abnormal relation between the length and the width of the bones are already present in the cartilaginous stage (fig. 2 *D* and *E* and fig. 4 *B*), and figure 4 A demonstrates a case of syndactylism in an entirely cartilaginous structure. It also indicates that the union of the basal phalanges 2 and 3 is probably due to a lack of separation into individual digits and not to fusion of already existing structures. Thus it can be stated that the abnormalities are to a certain extent already noticeable in the cartilaginous stage of the structures affected. It remains to be seen whether or not they date back to a still earlier stage of development.

EXPLANATION OF FIGURE 4

A, foot of an abnormal animal, showing syndactylism of the second and third basal phalanges (× 17).

B, leg of an abnormal animal, showing a normal femur and an abnormal tibia (× 17).

C, arm of an abnormal animal, showing a normal humerus and an abnormal radius and ulna (× 17)

It was suggested in the preceding communications [5] that lack of a nutritional factor may play a role in the production of the congenital malformations which occur in the offspring of female rats reared on diet 1. Subsequent experiments have supported this point of view. It has also been shown that the addition of small amounts of liver or of an alcoholic extract of liver to diet 1 can prevent the appearance of the congenital malformations described.[7] One may conclude from these experiments and from the histologic observations made in the present study that a nutritional factor absent in diet 1 but present in large amounts in liver is necessary for the normal prenatal bone development in these rats. Without this factor certain bones show arrest of development and malformations at the time of birth. That these malformations are not due merely to a disturbance of ossification is indicated in the sections which show developmental abnormalities already in the cartilaginous stage. In future experiments it is hoped to ascertain the time and the stage of embryonic development at which the primary injury to the developing organism is brought about by the lack of the postulated nutritional factor.

SUMMARY

A histologic study was made of some of the previously described skeletal abnormalities in the offspring of female rats reared on a deficient diet. The abnormal structures examined showed a more or less marked delay in ossification. The orderly relationship between the calcifying cartilage and the osseous parts of the bones was definitely disturbed. Persistence of cartilage was found in areas where ossification should have taken place. The cartilage itself showed signs of abnormal development. The outlines of the structures affected were abnormal in the cartilaginous stage. In early stages of development centers of calcification seen in atypical places explained the abnormal arrangement of the osseous trabeculae found in later stages. It is suggested that a nutritional factor absent in the deficient diet but present in large amounts in liver is necessary for the normal prenatal bone development in these rats.

Children's Hospital.

7. Warkany, J., and Nelson, R. C.: J. Nutrition **23**:321, 1942.

CLINICAL SIGNIFICANCE OF THE PATHOLOGIC CHANGES IN GIANT FOLLICULAR LYMPHADENOPATHY

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The comparatively newly discovered disease known as giant follicular lymphadenopathy has received widespread recognition by pathologists. It is beginning to attract the attention of clinicians, among whom, however, it is almost always mistaken for Hodgkin's disease. Clinically, the two diseases bear a strong resemblance to each other. Both are primarily diseases of the lymphoid system. Both are characterized by generalized, occasionally by localized, enlargement of the superficial lymph nodes and not uncommonly by splenomegaly. Histologically, the changes in the lymph nodes and the spleen are so widely divergent that confusion between the two diseases is scarcely possible.

In Hodgkin's disease the histologic picture is complex. The first observable change is to be found in hyperplasia of the lymph follicles (Longcope [1]; Symmers). These rapidly disappear and are replaced by diffuse hyperplasia of lymphocytes, distributed among which are variable numbers of large mononuclear cells together with multinuclear cells which are not to be distinguished from the megakaryocytes of the bone marrow. Eosinophils and eosinophilic myelocytes may or may not be present. At a later stage the lymphoid tissues are apt to show different degrees of connective tissue overgrowth. In a single node or even in several nodes the process may go on spontaneously to partial or almost complete replacement by dense, hyalinized bands or patches of connective tissue, which may obscure the earlier changes in such fashion as to render diagnosis difficult.

In giant follicular lymphadenopathy, on the contrary, the histologic changes are comparatively simple, consisting of numerical and dimensional hyperplasia of the lymph follicles (fig. 1). Cytologically, in many instances the follicles are distinguishable only with difficulty from those found in lymph nodes showing the familiar hyperplasia which occurs in

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From the laboratories of pathology of Bellevue, Fordham, Lincoln and Welfare hospitals of the Department of Hospitals, City of New York, and St. Francis Hospital, Peoria, Ill.

1. Longcope, W. T.: Bull. Ayer Clin. Lab. Pennsylvania Hosp. **1**:4, 1903.

association with innumerable inflammatory lesions and not uncommonly with neoplasms, both benign and malignant. At other times all the follicles in giant follicular lymphadenopathy are made up of essentially the same sorts of embryonal cells, that is to say, cells with very large hypochromatic or almost achromatic nuclei and sharply defined, often indented nuclear membranes, the so-called shadow cells, and similar but

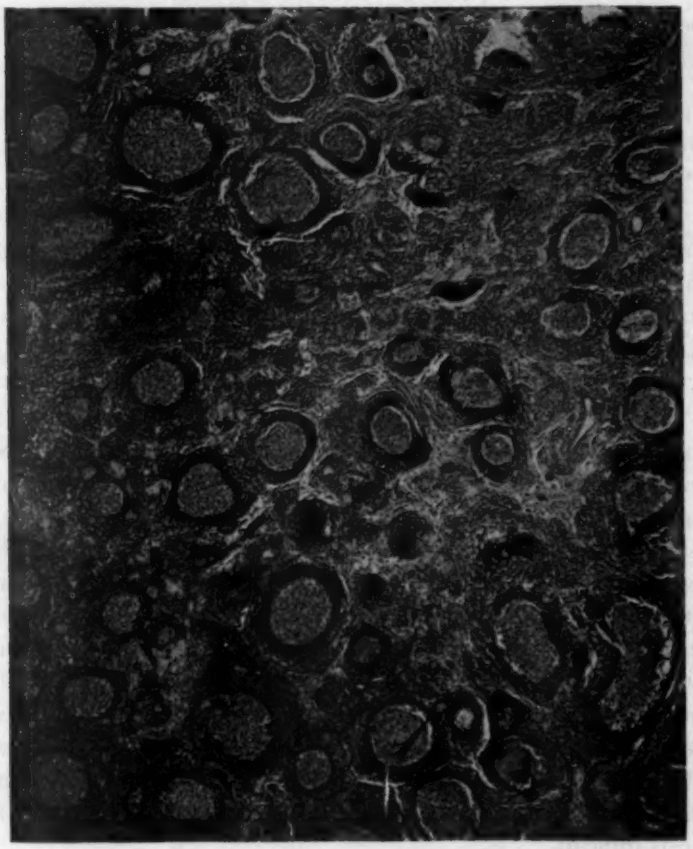

Fig. 1.—Very low power photomicrograph showing: numerical and dimensional hyperplasia of the lymph follicles in giant follicular lymphadenopathy, each with its limiting zone of small lymphocytes; distortion of follicles; interfollicular fibrosis.

slightly smaller cells which are somewhat richer in chromatin and the nuclear membrane of which is also indented in such fashion as to give the nuclei curious shapes. The latter cells are believed to be transitional forms between the very large shadow cells and the still smaller, richly

chromatic cells of the large lymphocytic type with no detectable limiting membrane and irregular, sometimes angulated nuclei. These cells of several types often lie in a filmlike syncytium and may be present in widely varying numbers (fig. 2). In certain instances the peripheral zone of small lymphocytes is lacking. In still others the follicles may be made up practically exclusively of small lymphocytes. As long as these

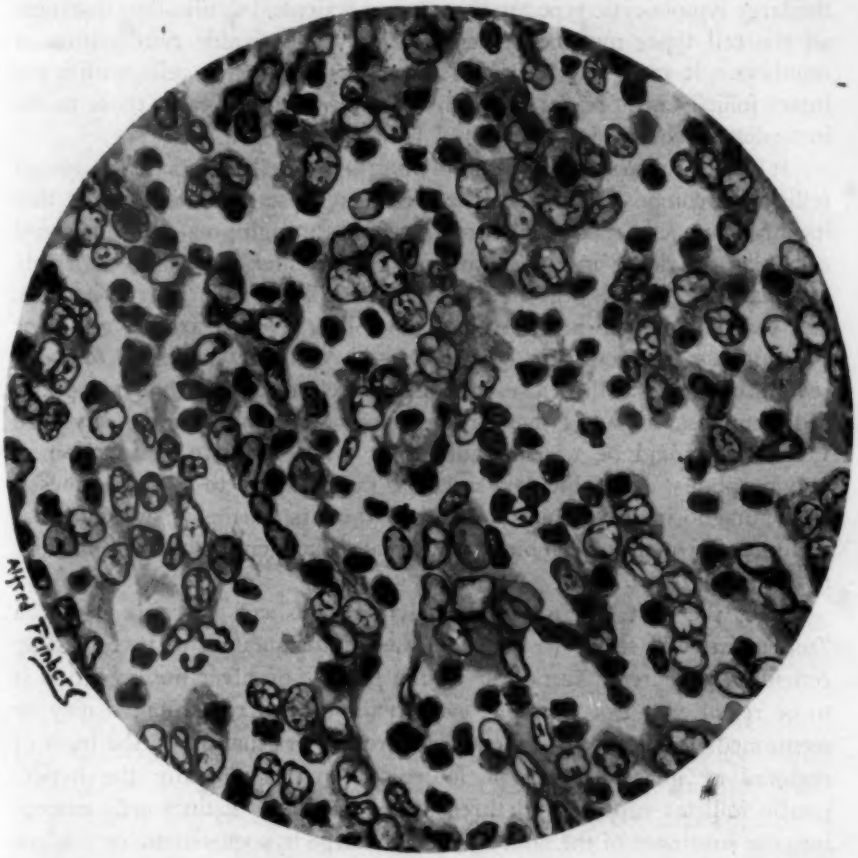

Fig. 2.—Oil immersion drawing to illustrate cell types in the polymorphous cell sarcoma following neoplastic transformation of the lymph follicles in giant follicular lymphadenopathy: large hypochromatic or shadow cells; smaller, slightly chromatic transitional shadow cells, and hyperchromatic cells of the large lymphocytic type, all lying in a syncytial matrix.

hyperplastic and variously modified collections of cells remain localized within the lymph follicles, the disease falls under the heading of giant follicular lymphadenopathy, but when the follicles rupture and the cells

escape into the surrounding tissues of the lymph node, the disease giant follicular lymphadenopathy is transformed directly into polymorphous cell sarcoma.[2]

The cells of polymorphous cell sarcoma may vary numerically within wide limits. In some instances the embryonal large hypochromatic cells, including the slightly smaller hypochromatic transitional forms, may predominate, and in other instances the relatively richly nucleated cells of the large lymphocytic type are the most prevalent. In still other instances all the cell types may be present in every conceivable combination of numbers. It may be stated as a general rule that the cells within the intact follicles are present in much the same proportions as those in the immediate vicinity of the ruptured follicles.

It may be assumed that polymorphous cell sarcoma of lymph follicles is composed of immature lymphocytes in view of the fact that its large hypochromatic cells are traceable through smaller transitional cells slightly richer in chromatin into still smaller but relatively richly chromatic cells which bear an arresting resemblance to large lymphocytes, and that all of them are derived from follicles which are set aside for the purpose of producing lymphocytes. A tumor made up of these several cells might, therefore, be appropriately designated polymorphous cell lymphosarcoma. However, in view of the uncertainties involved I think it would be wise to adhere to the descriptive designation of polymorphous cell follicular sarcoma rather than to commit oneself to the diagnosis of lymphosarcoma until there is conclusive evidence that the unit of growth is a lymphocyte, no matter how enticing the probability may appear to be.

That the unit of growth in polymorphous cell sarcoma is not a "reticulum cell" seems to be established if the method of investigating reticulum and "reticulum cells" by the process of silver impregnation is to be relied on. For example, even at the cost of repetition, it may be mentioned that in polymorphous cell sarcoma originating on the basis of regional or generalized giant follicular lymphadenopathy the hyperplastic follicles rupture and three morphologically distinct cells emerge into the substance of the nodes—the very large hypochromatic or shadow cells, the smaller hyperchromatic cells of the large lymphocytic type and the relatively large but poorly chromatic transitional cells. None of these cells is traceable to the argyrophilic reticulum fibers of the node—none is provided with fibrils which respond to special stains for reticulum cells, although both fine and coarse argyrophilic reticulum fibers may be brought out with clarity in the follicles and in other parts of the node. From these histologic considerations it is evident that the conception of sarcoma of lymph nodes composed of argyrophilic reticulum

2. Symmers, D.: Arch. Path. **26**:603, 1938.

cells is not tenable, since there is no scientifically established basis for the belief that an argyrophilic reticulum cell exists in the lymph follicle.

Giant follicular lymphadenopathy, in its earlier stages at least, is naturally inclined in the direction of benignancy, and spontaneous diminution in the size of the nodes or apparently complete disappearance may occur. Sometimes this may happen even late in its course. Recurrence, however, is not uncommon in either circumstance. In these respects Hodgkin's disease and giant follicular lymphadenopathy again resemble one another clinically. However, in the matter of radiation therapy giant follicular lymphadenopathy presents a noteworthy deviation from Hodgkin's disease in that it is susceptible of rapid alleviation if not cure, provided radiation is given at proper intervals and in appropriately small doses (Rubenfeld [3]). Indeed, in giant follicular lymphadenopathy the quick response of the lymph nodes to mild roentgen therapy is so impressive that if at a later date recurrence is noted and readjustment of the enlarged nodes is delayed or incomplete or both, and larger doses are required, one would seem to be justified in suspecting that the condition is undergoing some form of mutation (Symmers [2]).

PATHOLOGIC ANATOMY OF GIANT FOLLICULAR LYMPHADENOPATHY

Knowledge of the deeper changes in giant follicular lymphadenopathy is extremely scant, and the little that pathologists know of the disease as a whole has been made available practically exclusively by studies of excised superficial lymph nodes and a few spleens removed at operation. As far as I am aware, only one complete necropsy report has thus far appeared in the literature on the subject. Two additional reports are presented in this paper. Even from what is known at the present moment it is probably safe to concede that the disease is primarily inflammatory rather than neoplastic. However this may be, the fact remains that the unit of the disease is the hyperplastic lymph follicle and that hyperplasia of lymph follicles may be observed sufficiently often in certain diseases of the lymphoid system to indicate a fundamental phase in the evolution of these conditions, including, among others, giant follicular lymphadenopathy, Hodgkin's disease, lymphosarcoma and lymphoid leukemia, and that the ultimate clarification of these diseases is to be sought in the cause of hyperplasia of the lymph follicles.

In certain situations, notably the gastrointestinal tract, the geographic intimacy between the lymphoid tissues and an absorption surface of enormous dimensions would seem to indicate that the lymphoid cells are placed there for purposes of filtration, not alone of particulate substances but also of substances in solution or suspension. The lymph nodes throughout the body and the lymph follicles in the spleen, if one may judge from their location at strategic points, are called on to perform

3. Rubenfeld, S.: Am. J. Roentgenol. **64**:875, 1940.

a similar function. Beyond this simple fact nothing seems to be known of the function of the lymphocyte, either individually or collectively. In addition to the great lymphoid depots, I have pointed out that there is an auxiliary lymphoid system to be found in the form of small islands of lymphoid cells of wide distribution in the connective tissue framework of the thyroid, prostate, lungs, kidneys, liver, bone marrow and adrenals, in the subcutaneous fat tissues, in the mesentery and omentum and elsewhere.[4] These depots are often so small as to escape notice in the routine histologic examination of tissues; at other times they are large, sometimes provided with germinal centers, and thus easily seen. One is apt to receive the impression, I think, that the function of these lymphoid foci is even less clear than that of obviously identical cells in the gastrointestinal tract, lymph nodes and spleen, since they do not seem to be strategically placed for purposes of filtration. However, they not infrequently are brought into prominence in certain diseases. For example, in typhoid fever those in the liver commonly undergo slight increase in size and give rise to multiple so-called focal necroses. In the thyroid glands removed from patients with exophthalmic goiter lymphoid deposits with or without germinal areas are objects of almost daily observation. They are of common occurrence in prostates; here they are sometimes in the form of rounded, densely packed collections of small lymphocytes; at other times germinal follicles may be observed in them. That they are of clinical significance is indicated by the fact that there are instances recorded in which these cell collections have proliferated to form lymphosarcoma. At Bellevue Hospital I have had occasion to study a case of lymphosarcoma of the prostate arising in a man 30 years of age.[5] At necropsy the prostate was replaced by a moundlike growth measuring 9 by 7 cm. The walls of the bladder were extensively infiltrated, as were the psoas muscles on both sides, the ureters and kidneys, the tissues around the adrenal capsules and the perilobular connective tissues of the liver. Coupland[6] recorded an almost identical finding in a man 27 years of age.

Since the lymph follicle is a prerequisite for the development of giant follicular lymphadenopathy, it follows as a matter of no great surprise that, although giant follicular lymphadenopathy has been subjected to postmortem examination on three occasions only, in each instance hyperplastic lymphoid foci with or without germinal centers have been demonstrated in practically all of those organs which belong to the auxiliary lymphoid system. Giant follicular lymphadenopathy is not a disease which is necessarily restricted to the lymphoid system proper; its ramifications may include any organ in the body which is provided with auxiliary deposits of lymphoid cells.

4. Symmers, D.: Arch. Int. Med. **4**:218, 1909.
5. Symmers, D.: Arch. Surg. **6**:755, 1923.
6. Coupland, S.: Tr. Path. Soc., London (1876-1877) **28**:179, 1877.

In a previous publication [2] I described a case of giant follicular lymphadenopathy of eight years' duration which terminated in acute necrotic folliculitis of the lymph nodes and spleen. In the same paper 7 cases of Hodgkin's disease were included. Four of the 7 cases were investigated at necropsy, and 3 of the 4 showed the extraordinary combination of giant follicular lymphadenopathy, polymorphous cell sarcoma of the lymph follicles and Hodgkin's disease. Necropsy in the remaining case showed combined giant follicular lymphadenopathy and Hodgkin's disease but no signs of polymorphous cell follicular sarcoma. In the remaining 3 cases only excised superficial lymph nodes were available for microscopic examination. In 2 of these 3 cases giant follicular lymphadenopathy, polymorphous cell follicular sarcoma and Hodgkin's disease were found in excised lymph nodes from both patients. In the remaining case the combination of the same three diseases was found in two sets of lymph nodes removed twenty months apart.

In the same paper 4 cases were described in which giant follicular lymphadenopathy and lymphoid leukemia were associated and 7 cases in which the hyperplastic follicles of giant follicular lymphadenopathy had ruptured, resulting in their transformation into polymorphous cell sarcoma. From these few facts alone it is evident that the histologic variations of giant follicular lymphadenopathy are more intricately involved than those of any other known disease of the lymphoid system. In the present paper it is my purpose to add 6 cases bearing on the pathology of giant follicular lymphadenopathy, including two reports on the necropsy observations, and a case of predominant if not primary giant follicular lymphadenopathy of the spleen.

Terplan,[7] in 1929, contributed a case of giant follicular lymphadenopathy under the heading "A Peculiar Granuloma-like Systemic Disease." Necropsy was done, and the findings in the deeper viscera are believed to be the first recorded in the literature on the disease.

Terplan's case was that of a white woman 61 years of age who died about nine months after the onset of an illness characterized by emaciation, dyspnea followed by orthopnea, edema of the lower extremities and of the region of the sacrum, generalized enlargement of the superficial lymph nodes and massive enlargement of the spleen. At necropsy the cervical nodes were found to be about "the size of a bean," as were most of the axillary and supraclavicular nodes, although occasional ones reached the proportions of a "small prune." The superficial and deep inguinal nodes were from "bean to hazelnut size," occasionally "the size of a prune." The retroperitoneal nodes were greatly enlarged, some of them forming clusters the "thickness of one's finger"; others were discrete and ranged in size from that "of a cherry to that of a hazelnut." The spleen measured 20 by 13 by 8 cm. and showed innumerable grayish follicles.

Histologic examination revealed numerous greatly enlarged, pale-staining lymph follicles in all the nodes, superficial and deep, and in the interstitial tissues of

7. Terplan, K.: Verhandl. d. deutsch. path. Gesellsch. **24**:65, 1929.

the liver, the bone marrow and the spleen. Most of the follicles were isolated, others were confluent and still others formed curious arrangements—clover leaf, dumbbell or kidney shaped. The lymphoid collections in the liver and the bone marrow appeared to me to represent hyperplasia of cells belonging to the auxiliary lymphoid system.

In Terplan's case the microscopic changes were limited to the lymph nodes, spleen, liver and bone marrow. At St. Francis Hospital, Peoria, Ill., a second and similar case was investigated at necropsy by Dr. E. J. Kraus, and the findings were almost exactly like those described except that in Kraus's case the lymph nodes, spleen, liver, kidneys, the mesenteric and omental fat and the peritoneum were involved, while the bone marrow was intact. Dr. Kraus has generously allowed me to make full use of his data for presentation in this paper.

Case 1.—A man aged 58, white, American, was admitted to St. Francis Hospital, Peoria, Ill., June 22, 1940 and died June 23. The clinical diagnosis was lymphosarcoma. The attending physician was Dr. H. M. Wilson.

The patient complained of dyspnea and progressive increase in the size of the abdomen together with swelling of the ankles and cough. He stated that he had lost 20 pounds (9 Kg.) in weight in the past four years and that he had been subject to night sweats for several months prior to admission. Physical examination revealed a well developed and well nourished white man who was moderately dyspneic and whose temperature, pulse and respiration were normal. The cervical, axillary and inguinal nodes were increased in diameter to the extent of 2 to 3 cm. and were firm in consistence, discrete and nontender. The abdomen was markedly distended; the skin covering it, tense. There was slight pitting edema together with a marked fluid wave. There were no palpable masses. Both ankles showed slight pitting edema. The blood count was 3,990,000 red cells and 13,200 white cells; the hemoglobin content was 78 per cent. The differential count revealed 62 per cent polymorphonuclear neutrophils, 10 per cent transitional cells, 6 per cent eosinophils, 20 per cent lymphocytes and 2 per cent monocytes.

Necropsy (Dr. E. J. Kraus) was done one hour post mortem. The body was that of an emaciated man 177 cm. in length. There was generalized edema of the subcutaneous tissues especially of the scrotum, legs and feet. The abdomen was markedly distended by fluid. When the abdomen was opened, many liters of clear yellowish fluid were removed. Each pleural cavity contained about a liter of fluid of the same sort. The pleura on both sides was reddish white and markedly thickened. The lungs showed nothing worthy of note in the present connection. The tracheobronchial lymph nodes were enlarged, anthracotic and edematous. The heart was somewhat smaller than normal, the left ventricle slightly dilated, the heart muscle brownish, the valves apparently sufficient and the coronary arteries and aorta well preserved. The liver was enlarged, weighing 2,750 Gm. It was brownish and firm in consistency and scattered throughout were many small flat whitish tumor-like infiltrations, the largest approximating 1 cm. in diameter. The spleen was enlarged and weighed 900 Gm. It was rather soft, and the pulp was reddish. The follicles were grayish white, extremely numerous and closely apposed one to the other. The kidneys were normal in size but slightly congested and showed many whitish or grayish white areas resembling tumor tissue, some of which were rounded or oval and well defined, while others varied in shape, with borders obscured. These areas differed in size, the largest reaching about 5 cm. in its longest diameter.

The mesentery was thickened, firm in consistency and widely infiltrated by whitish tumor-like tissue. The mesenteric lymph nodes were numerous and enlarged to the size of 3 or 4 cm. They were soft and grayish or grayish white. The retroperitoneal lymph nodes were discrete but closely packed and formed an enormous mass, in which the largest nodes measured from 3 to 5 cm. in diameter. The hypogastric and the anterior inguinal nodes were similarly involved and whitish, and varied in diameter from 4 to 5 cm. The lymph nodes in the region of the hilus of the liver, the perigastric and the pancreatic lymph nodes and those at the hilus of the spleen were enlarged, whitish, smooth, glistening and diffusely infiltrated by whitish tumor-like tissue. The largest among them were about 4 cm. in diameter. The parietal peritoneum was irregularly thickened, whitish and covered by tumor-

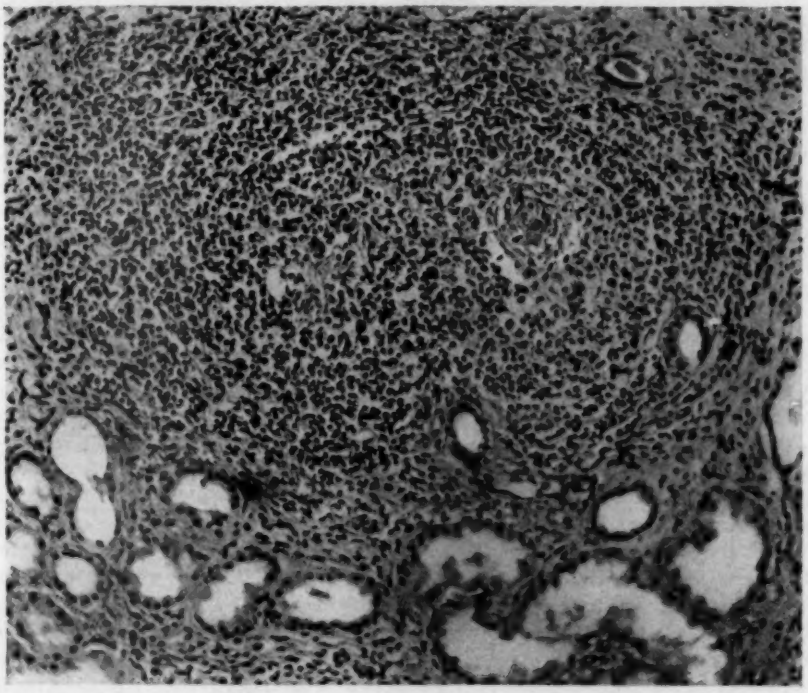

Fig. 3 (case 1).—Photomicrograph of kidney showing the type of lymphoid focus encountered in the several organs belonging to the auxiliary lymphoid system. From a case of giant follicular lymphadenopathy; hematoxylin and eosin; paraffin; × 175.

like nodules measuring from 0.5 to 1.5 cm. in their longest diameters. The rest of the organs, including the adrenals, urinary bladder, prostate and bone marrow, showed nothing worthy of mention in the present connection.

Microscopic examination of the enlarged lymph nodes throughout the body showed much the same appearance. The lymph follicles were for the greater part massively enlarged; some were rounded or oval and others elongated, sometimes assuming fantastic designs simulating such shapes as those of a boat, hook and hourglass. Practically all of them were surrounded by a zone of small lymphocytes of variable

thickness. The germinal centers were occasionally made up almost exclusively of small lymphocytes, but for the greater part they stained lightly, were loosely packed and consisted of a mixture of moderately well nucleated cells of the large lymphocytic type, considerable if not preponderating numbers of large embryonal hypochromatic cells and a sprinkling of small lymphocytes. The spleen was riddled with hyperplastic lymph follicles consisting mostly of small lymphocytes. In them an occasional germinal area was to be seen. Scattered through the interstitial tissues of the kidney, in both the cortex and the medulla, were great numbers of deposits of small lymphocytes (fig. 3). They assumed variable shapes and sizes, and many of them communicated one with another to form collections large enough to occupy one half or more of the low power field of the microscope. In a noticeably large number of instances lightly staining germinal areas stood out prominently, but around them a sharply defined peripheral lymphocytic zone was often lacking. In the liver, particularly in the interlobular interstitial tissues but not infrequently among the liver cells themselves, were great numbers of relatively small collections of closely packed lymphocytes. No germinal follicles were observed in them. In the fatty tissues of the omentum there were extraordinarily large numbers of hyperplastic lymphoid collections, without a peripheral limiting lymphocytic zone; others were marked by lightly staining germinal follicles. The lymphoid tissues of the lower portion of the small intestine were hyperplastic to a marked degree, and germinal follicles were numerous. The lymphoid collections in the liver, the kidney and the fat tissues of the omentum are best accounted for, I believe, on the basis of hyperplasia of cells belonging to the auxiliary lymphoid system.

Case 2.—A white woman aged 74 years died in Welfare Hospital Feb. 28, 1941. Two years prior to admission she noticed that her abdomen was enlarging. She was admitted to Kings County Hospital, and several abdominal paracenteses were done. The fluid was milky in appearance. A lymph node was removed at Kings County Hospital, and microscopic examination by Dr. W. W. Hala revealed the presence of giant follicular lymphadenopathy (fig. 4*A*). At the time of admission to Welfare Hospital there was evidence of marked loss of weight and the patient was extremely weak. The abdomen was protuberant, and the abdominal wall and both lower extremities were edematous. The cervical lymph nodes on both sides were palpable, discrete and measured from 0.5 to 2 cm. in diameter. The axillary lymph nodes on both sides were similarly enlarged. Biopsy of a second lymph node also revealed the presence of giant follicular lymphadenopathy. While she was in the hospital abdominal paracentesis was done and 5,000 cc. of milky white fluid removed. Numerous blood counts were made. The red blood cells averaged 4,800,000, the hemoglobin content 86 per cent and the white blood cells 8,000. The differential count showed polymorphonuclear neutrophils 68 per cent, lymphocytes 23 per cent, eosinophils 3 per cent and monocytes 6 per cent. No abnormal cells were detected.

At necropsy (Dr. Julius Rosenthal) there was extensive edema of both lower extremities. The abdomen was protuberant, and on section 4,000 cc. of chylous fluid was removed. The mesentery of the small intestine was thickened to the extent of about 3 cm., and on section through it there were innumerable whitish areas together with enlarged lymph nodes, which were grayish, succulent and, on section, bulging. The liver weighed 3,300 Gm. Scattered throughout were nodules, which varied in diameter from 1 to 10 cm. Approximately four fifths of the liver was replaced in this fashion. The nodules were whitish and somewhat rubbery. The contents bulged markedly over the cut edges. The pancreas was embedded in whitish tissue presenting the same general characteristics as that just

described. The spleen weighed 160 Gm. and on section revealed no changes visible to the naked eye.

The abdominal lymph nodes were enlarged to an enormous degree. The abdominal aorta, the iliac arteries and the inferior vena cava were embedded in

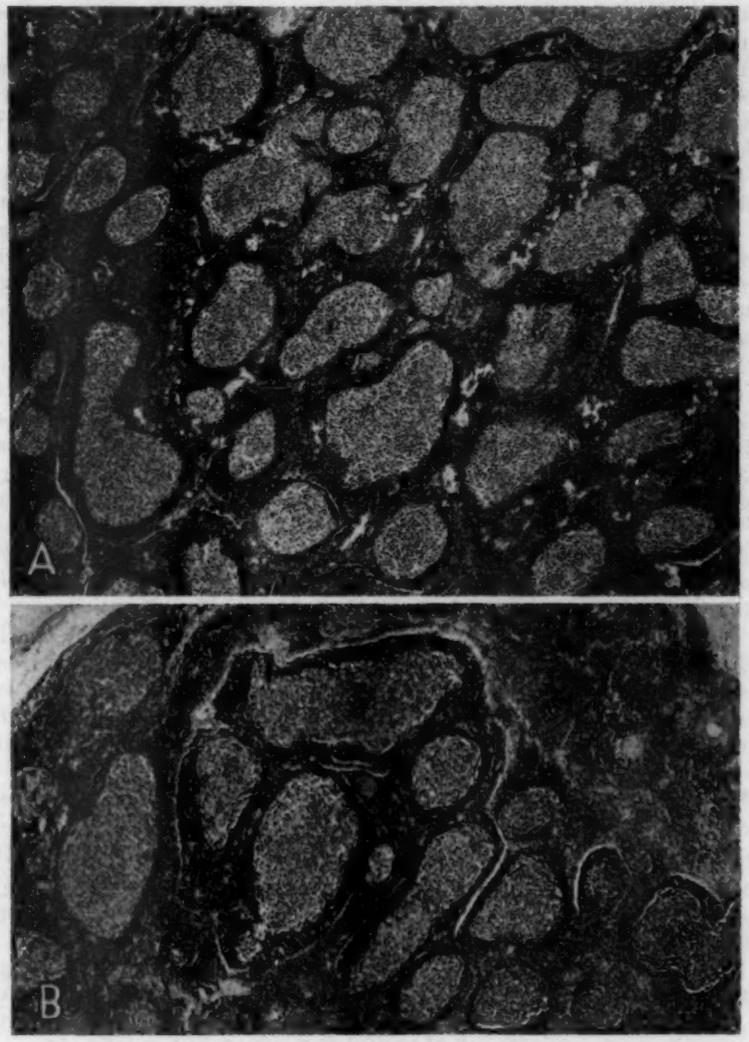

Fig. 4 (case 2).—*A*, photomicrograph showing numerical and dimensional hyperplasia and distortion of lymph follicles. From an inguinal lymph node removed two years before death in a case of giant follicular lymphadenopathy; hematoxylin and eosin; paraffin; × 14. *B*, photomicrograph showing numerical and dimensional hyperplasia and distortion of lymph follicles in a retroperitoneal lymph node removed at necropsy, two years after onset of giant follicular lymphadenopathy. (Compare with *A*.) Hematoxylin and eosin; paraffin; × 14.

masses of closely packed lymph nodes measuring on the average about 3 cm. in their longest diameters. These nodes were whitish, rubbery and bulged when sectioned. In still other areas the lymph nodes were enlarged but discrete.

Microscopic examination of a lymph node removed from the inguinal region during life showed complete replacement by enormously hyperplastic follicles. Some of them were rounded or oval, others showed various fantastic outlines and many of them were fused. Each was surrounded by a zone of small lymphocytes. The germinal areas consisted of moderately richly chromatic cells of the large lymphocytic type and embryonal hypochromatic or shadow cells in like numbers. There was, in addition, a sprinkling of small lymphocytes. The interfollicular tissues were richly infiltrated by small lymphocytes.

Microscopic examination of an enlarged lymph node removed from the axilla during life showed almost identical changes, that is to say, complete replacement by hyperplastic lymph follicles, each of which was surrounded by a zone of lymphocytes enclosing light-staining germinal areas made up of fairly richly chromatic cells of the large lymphocytic type and embryonal hypochromatic shadow cells in about equal proportions. The follicles were often so closely packed as almost to obliterate the interfollicular spaces.

Microscopic examination of lymph nodes removed at necropsy from various parts of the body revealed an almost identical picture: innumerable well preserved hyperplastic lymph follicles, most of which were extraordinarily large (fig. 4 *B*) and were circumscribed by zones of tightly packed small lymphocytes. The germinal areas were composed of loosely arranged cells, most of which were of the embryonal large lymphocytic type; others were small lymphocytes. The lymph follicles were, for the greater part, closely set together, and the interfollicular spaces consisted of densely packed small lymphocytes. In still other instances there were only the remains of germinal follicles. These were scattered through the substance of the node but were still easily recognized as follicular remnants.

The kidney showed, lying in the interstitial spaces of both the cortex and the medulla but predominantly in those of the medulla, numerous collections of lymphocytes of various shapes and sizes. Occasionally a large lymph follicle was found consisting of a peripheral zone of thickly packed small lymphocytes and a germinal center made up of cells of the embryonal large lymphocytic type with a more or less rich admixture of smaller lymphocytes.

The liver showed fairly numerous small and moderately large collections of closely packed lymphocytes. Most of them lay in the interstitial tissues, but some were located in the parenchyma itself. No lymph follicles were found. There was no indication of intercellular invasion. In the liver, as in the kidney, the lymphoid collections seemed to be best interpreted as hyperplasia of preexisting foci belonging to the auxiliary lymphoid system.

In the subepithelial tissues of the sigmoid were a few scattered small collections of lymphocytes, but occasionally a very large lymph follicle was to be observed, the histologic characteristics of which differed in no essential from those already described in the lymph nodes; that is to say, there was a circumferential zone of closely packed small lymphocytes surrounding thinly scattered cells of the large and small lymphocytic type.

The lymph nodes in which the pancreas was buried showed the same changes as those described elsewhere, but there was no indication of invasion of the pancreas itself. The same was true of the left adrenal capsule and of the celiac ganglion.

These 3 cases (Terplan, Kraus, Symmers) prove, I believe, that giant follicular lymphadenopathy may exist as an independent disease

characterized by numerical and dimensional hyperplasia of the lymph follicles of the lymph nodes or of the lymph nodes and the spleen and by hyperplasia of minute lymphoid foci normally resident in the interstitial tissues of almost every organ in the body, the latter constituting what I have ventured to describe as the "auxiliary lymphoid system." In case 2, for example, the disease is shown to have maintained its individuality for a period of at least two years as judged by the histologic identity of lymph nodes removed at the approximate date of onset of the disease and those removed after death. By this I do not mean to imply, of course, that giant follicular lymphadenopathy always maintains its individuality from onset to death. On the contrary, it is shown in this paper that it may often change its character entirely.

Case 3.—A white man aged 48 years was admitted to Fordham Hospital April 26, 1940 and died May 12. He complained of weakness and pains in the legs of eight months' duration and of inability to walk. He stated that he had lost about 50 pounds (22.5 Kg.) in weight since the onset of illness. Physical examination revealed atrophy of the muscles of the lower extremities. A consultant in neurology made the following note: "Facial and lingual innervation normal. Deep reflexes of right upper extremity absent. Paresis of left lower extremity with wasting, particularly of anterior thigh group. Both knee jerks absent. Left ankle jerk absent. No Babinski sign. Marked tenderness over nerve trunks, especially on the left. Hypalgesia of glove and stocking type present bilaterally. Abdominal reflexes active. Comment: The foregoing evidence indicates involvement of the cord and peripheral nerves." Enlarged lymph nodes were palpated in both the axillas and the groins. Roentgen examination of the chest showed indications of a thickened pleura in the region of the axilla on the left side. The scapulas, the outer ends of both clavicles and the lesser and greater trochanters of both femurs showed areas of "osteoporosis and osteosclerosis suggesting metastasis," together with erosion of the lesser trochanter of the right femur and an incomplete pathologic fracture of the trochanter. The lumbar vertebrae showed small areas of bone destruction. There was a pathologic fracture of the wing of the ilium adjacent to the upper part of the right sacroiliac articulation. The differential white blood cell count showed 62 per cent polymorphonuclears, 34 per cent lymphocytes and 4 stabs. On the sixteenth day after admission the patient suddenly died with symptoms indicating pulmonary embolism.

At necropsy (Dr. Louis Ferraro) there was marked axillary and inguinal adenopathy. When the chest was opened, enlarged lymph nodes were found lying on the upper surface of the diaphragm, in the anterior and the superior mediastinum, in the tissues surrounding the great vessels of the neck and in the axillary regions, where they were discrete.

The right pleural cavity contained 600 cc. of straw-colored fluid. The pleura on both sides was irregularly thickened owing to the presence of numerous smooth nodules lying in the parietal layer. These nodules were most prominent in the lower portion of the pleura and, it was estimated, covered about 30 per cent of the entire pleural surface. Many of the pleural nodules replaced the intercostal muscles and infiltrated the adjacent ribs.

The heart appeared to be well preserved except for a few whitish firm nodular infiltrations in the epicardium. The aorta and the inferior vena cava lay in a bed of enlarged lymph nodes, which were whitish and adherent to one another in the

region of the lower lumbar vertebrae, where the individual nodes measured up to about 5 cm. in thickness. The external iliac and femoral arteries were similarly surrounded by enlarged nodes, and the psoas major muscles were extensively infiltrated by whitish tumor tissue. The lower lumbar vertebrae were largely replaced by whitish tumor infiltrate, as were the ilia, the lateral portions of both clavicles and the trochanter of the right femur.

The spleen weighed 195 Gm. On section the pulp was reddish brown. At the upper pole was a circumscribed grayish white firm nodule which was raised above the surface to the extent of 1.5 cm. Throughout the rest of the spleen there was

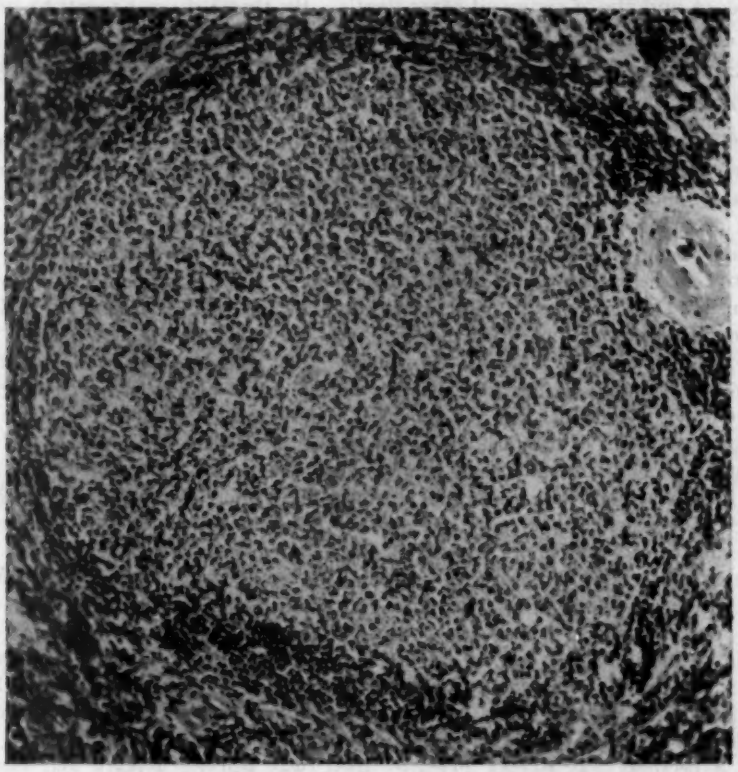

Fig. 5 (case 3).—Photomicrograph showing an enormous lymph follicle in spleen, composed of richly nucleated embryonal cells of the large lymphocytic type and embryonal hypochromatic or shadow cells. Note the small point of rupture near the lower right hand corner, with escape of follicular cells into the surrounding tissues. A central artery is seen near the right upper corner. From a case of giant follicular lymphadenopathy; hematoxylin and eosin; paraffin; × 153.

noticeable hyperplasia of the malpighian corpuscles, many of which were markedly increased in size.

The cortex of the left adrenal showed a circumscribed nodule which measured 1.5 cm. in width and 6 mm. in thickness. On stripping the capsule of the left kidney a flattened whitish nodule was visible on its posterior-superior surface.

This nodule measured 1.5 cm. in width. The kidney bulged in the region of the pelvis, and on section it was found that the upper portion of the pelvis was almost completely replaced by a whitish tumor which projected into the medulla for a distance of 1 cm. and invaded the peripelvic fat.

None of the remaining organs showed anything worthy of record in the present connection.

Microscopic examination showed that many if not the majority of the lymph follicles in the spleen were markedly hyperplastic, some of them almost completely filling the low power field of the microscope (fig. 5). As a rule they were rounded

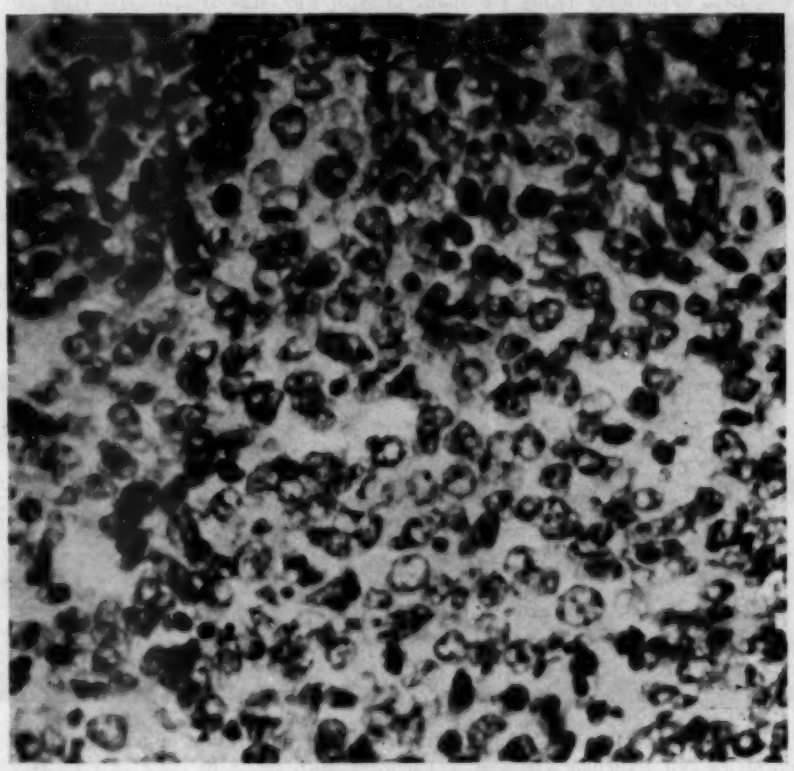

Fig. 6 (case 3).—Photomicrograph showing infiltration of the substance of the spleen by embryonal large hypochromatic or shadow cells and cells of the embryonal large lymphocytic type in about equal proportions. The picture illustrates the transformation of giant follicular lymphadenopathy into polymorphous cell sarcoma of lymph follicles. (See fig. 5 in same case.) Hematoxylin and eosin; paraffin; × 588.

or oval, and the pulp between them was congested. Many, however, of the hyperplastic follicles were kidney or hook shaped or otherwise thrown into fantastic forms. Practically all of them were surrounded by an unbroken layer of small lymphocytes. The centers of the follicles stained lightly and consisted of about equal numbers of large rounded, oval or elongated nuclei which contained moderate

quantities of chromatin and of smaller numbers of larger nuclei which were only slightly provided with chromatic particles—the so-called shadow cells. In other parts of the spleen there was no evidence of follicle formation but the parenchyma was flooded with a mixture of the two types of cells just described, that is to say, large moderately richly chromatic cells of the large lymphocytic type and somewhat larger, sharply defined cells whose chromatic content was almost negligible—the so-called shadow cells (fig. 6).

Lymph nodes removed from various parts of the body were examined microscopically. Some of the nodes were completely replaced by rounded, oval or elongated, moderately richly chromatic cells of the large lymphocytic type and a scattering of larger rounded or occasionally indented nuclei which were free from

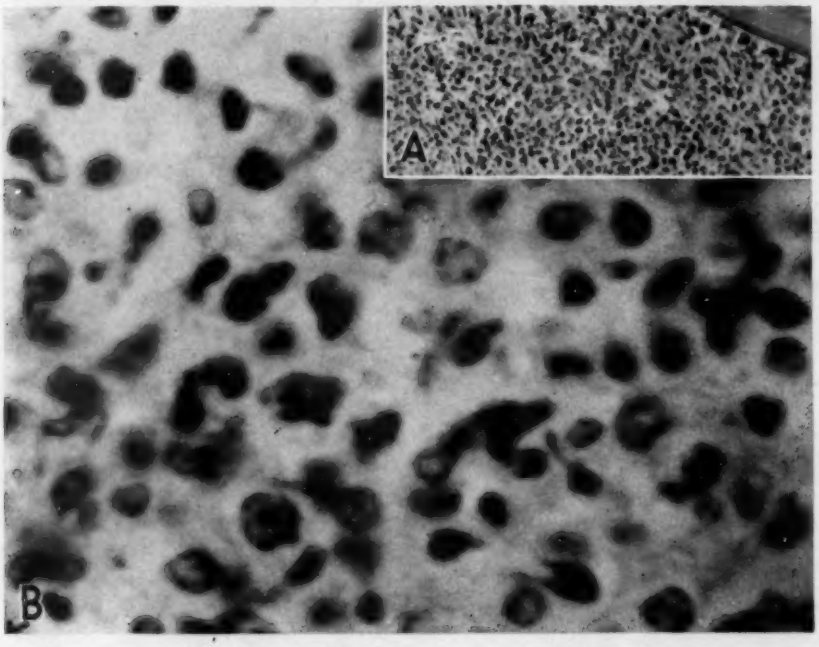

Fig. 7 (case 3).—*A*, photomicrograph showing infiltration of marrow in polymorphous cell follicular sarcoma. (Compare with fig. 5 in same case.) Hematoxylin and eosin; paraffin; × 157. *B*, photomicrograph of marrow showing infiltration by embryonal cells of the large lymphocytic type and of embryonal hypochromatic or shadow cells in approximately the same proportions. (Compare with fig. 6 in same case.) Hematoxylin and eosin; paraffin; × 1104.

chromatin. These cells were supported by a delicate stroma of connective tissue. In still other nodes remnants of lymph follicles were present, and in many instances they were bordered by a delicate, broken connective tissue membrane, which was infiltrated by variable numbers of closely packed small lymphocytes. The centers of the lymph follicles were composed of the same types of cells as those just described, and in some instances the points of rupture of the follicular membrane could be seen. From the points of rupture the cells could be traced directly into the substance of the nodes.

Microscopic examination of bone marrow showed complete replacement of the interlamellar spaces by the overgrowth of an admixture of cells, prominent among which were richly nucleated cells of the large lymphocytic type (fig. 7 *A*). Scattered among these were about equal numbers of larger, poorly nucleated shadow cells (fig. 7 *B*). The cells were separated at intervals by a delicate stroma of pinkish-staining connective tissue. The lung, the parietal pleura and the medulla of the adrenal capsule were similarly infiltrated.

Microscopic examination of the rest of the organs, including those of the gastrointestinal tract, and the liver, kidneys, prostate, testes and pancreas, showed no changes of interest in the present connection.

Polymorphous cell sarcoma derived directly from the hyperplastic lymph follicles of giant follicular lymphadenopathy is divisible into two anatomic groups—one in which the sarcomatous changes are confined to the lymphoid tissues, preponderantly the lymph nodes, while the spleen may or may not be involved, and a second group in which the changes in the lymph nodes and spleen are accompanied by similar alterations in organs not customarily included in the lymphoid system, among them the lungs, pleura, kidneys and adrenals, and by widespread destructive lesions of bone, giving rise to spontaneous fractures. An example of the latter type of dissemination has just been described and is believed to be the first of its sort thus far recorded.

Case 4.—A white man aged 53 years, Polander, miner, was admitted to Bellevue Hospital July 3, 1940 and died August 5. The patient complained of cough, weakness and loss of appetite. Examination revealed tubercle bacilli in the sputum and extensive signs of tuberculosis in both lungs. The superficial lymph nodes, including those of the neck, axillas, epitrochlear and inguinal regions, were markedly enlarged, discrete and freely movable. A lymph node was excised from an axilla for biopsy. The liver was palpated 2 cm. below the costal margin on the right side. The spleen was palpable. Throughout the patient's stay in the hospital the white blood cell count was low, varying between 3,000 and 5,000 per cubic millimeter, with a constant preponderance of polymorphonuclear leukocytes. No abnormal forms were detected at any time.

At necropsy (Dr. David Spain) the body was that of an emaciated white man. External examination revealed nothing worthy of note except that the lymph nodes in the cervical, axillary, epitrochlear and inguinal regions were enlarged. The nodes were discrete and freely movable and measured from 0.5 to 5 cm. in diameter. Enlargement of the lymph nodes was most pronounced in the inguinal regions.

When the abdomen was opened, the liver was found to extend 3.5 cm. below the costal margin in the midclavicular line; the spleen, 10 cm. below the left costal border. Both lungs showed widespread tuberculosis with cavitation. The liver weighed 1,900 Gm. and on section appeared to be well preserved.

There was marked generalized lymphadenopathy. The inguinal nodes were largest, measuring on an average 5 cm. in their longest diameters. They were of rubbery consistency; most of them were discrete, but a few were adherent to one another. On section they were homogeneous and grayish. The mesenteric nodes presented, for the greater part, a similar appearance, but some of them showed in addition caseous areas. In occasional instances small calcified nodes were noted.

Microscopic examination of an axillary lymph node removed during life showed a few follicular remnants. Within the broken follicles and streaming out

into the substance of the node were hordes of cells several times larger than the small lymphocyte. Some of these cells were richly nucleated; the others, which were still larger, were almost bereft of chromatin and presented a sharply defined nuclear membrane—the so-called shadow cells. In places the cells lay in a lightly staining smooth syncytial matrix.

Microscopic examination of the deep lymph nodes revealed an entirely different picture (fig. 8). In them the cells were diffusely distributed, loosely arranged and lay in a lightly staining smooth syncytium. Many of them were several times again as large as the small lymphocyte. As a general rule, they possessed deeply staining large rounded nuclei, and many of the nuclei were partially or

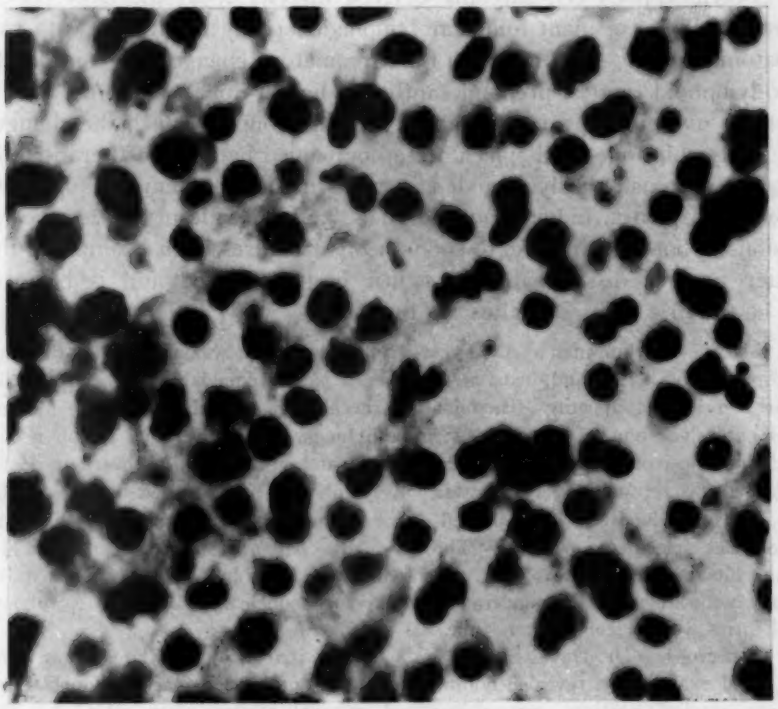

Fig. 8 (case 4).—Photomicrograph of a retroperitoneal lymph node showing very large immature cells of the large lymphocytic type lying in a syncytial matrix, some of them beginning to acquire cytoplasm from the syncytium. This case is believed to be one of lymph node sarcoma derived from overgrowth of younger forms of cells of the embryonal large lymphocytic type, commencing as polymorphous cell follicular sarcoma of the superficial lymph nodes. Hematoxylin and eosin; paraffin; × 1104.

completely surrounded by a more or less distinctly differentiated, lightly staining cytoplasm which not only bore a resemblance to the syncytial matrix but appeared to be identical with it.

In some of the lymph nodes epithelioid tubercles were present. Microscopic examination of the lungs showed widespread fibrocaseous tuberculosis. The spleen

was deeply congested and showed a fairly large number of necrotic epithelioid tubercles and numbers of atrophic lymph follicles. Microscopic examination of the rest of the organs showed nothing worthy of note in the present connection.

In this case the first changes to be observed were those of polymorphous cell sarcoma of a lymph node excised from an axilla during life. At necropsy, however, great numbers of enlarged nodes were found in the abdomen. They were completely replaced by cells which bore a resemblance to mature large lymphocytes. However, they differed from the mature large lymphocyte in several particulars: they were larger and were provided with a relatively abundant pale-staining smooth cytoplasm arranged either around the richly chromatic nuclei or at one side, and, moreover, they lay in a lightly staining smooth, syncytical bed which was structurally and tinctorially identical with their cytoplasm.

From these observations I am led to the conclusion that the preponderating cells in the enlarged nodes found at necropsy represented a more advanced developmental phase of those cells which I have described in this paper and elsewhere as embryonal hyperchromatic cells of the large lymphocytic type. In this case the disease, in my opinion, started as localized polymorphous cell sarcoma and ended as generalized sarcoma of lymph nodes composed of unusually large cells of the immature lymphocytic type.

CASE 5.—A white woman aged 57 years was first admitted to Bellevue Hospital Oct. 23, 1940 and was discharged November 18. About four and one-half years previously she had migratory pains in various joints, and one year before admission she began to notice lumps in her neck. These, she said, would come and go, and after several months she found similar lumps in both axillas and later in both inguinal regions. She also said that she had felt listless during the past year and had lost about 20 pounds (9 Kg.) in weight, and for several months before admission she was slightly short of breath on exertion. She also complained of intermittent backache, which was never very severe. Physical examination revealed generalized superficial lymphadenopathy involving the submaxillary, submental, cervical and inguinal nodes. The nodes were firm, discrete, freely movable and not tender. The spleen was palpated at the iliac crest on the left side. The blood counts showed an average of 4,250 white cells, of which 42 per cent were polymorphonuclear neutrophils and 58 per cent lymphocytes, large lymphocytes predominating in the proportion of about 2 to 1.

The patient was readmitted to Bellevue Hospital June 9, 1941 and died August 2. She complained of swelling of the right leg and of lumps in various parts of the body. Physical examination revealed massive enlargement of the cervical, axillary, inguinal and femoral nodes, all of which were nontender, firm and discrete. The spleen at this time was palpable 3 fingerbreadths below the costal margin. The blood count showed 2,680 white cells, of which 46 per cent were polymorphonuclear neutrophils and 54 per cent lymphocytes, large forms again predominating.

At necropsy (Dr. Margaret Bevans) the body was that of a well developed woman 58 years of age. External examination revealed no changes of note other than the presence of markedly enlarged lymph nodes in the axillary, inguinal and posterior cervical regions together with palpable nodes in both epitrochlear

regions and in the left popliteal region and a few shotty nodes in the left infraclavicular space. The abdomen was distended, and there was moderate edema of the ankles, particularly of the right one.

When the abdomen was opened, about 5 liters of cloudy fluid was removed. About 1,000 cc. of cloudy fluid was removed from the left pleural cavity and 200 cc. from the right. The liver was enlarged and weighed 2,250 Gm. Scattered through the parenchyma were innumerable whitish specks, the largest measuring 3 mm. in diameter. The spleen was enlarged and weighed 380 Gm. On section the substance was dark red and friable, and scattered through it were numbers of whitish foci.

In addition to large clusters of lymph nodes in both the axillary and the inguinal regions, enlarged nodes were present in the posterior mediastinum, at the hilus of both lungs, around the aorta, especially the abdominal portion, and in the regions of the pancreas, kidney and celiac axis. These surrounded and compressed the inferior vena cava and the aorta. Huge clusters of enlarged lymph nodes were present in the leaves of the mesentery. In the region of the brim of the pelvis was another large collection of lymph nodes, some of which were hemorrhagic, others necrotic. The great majority of the nodes were discrete and rubbery, and their cut surfaces were dead white.

Marked osteoporosis involved all of the ribs so that only a shell of bony tissue surrounded a grayish red spongy marrow. The body of the sternum was thinned but not nearly to the same degree as the ribs. No signs of compression of the vertebrae could be detected on examination from the interior of the body. Block sections of the twelfth dorsal to the fifth lumbar vertebrae were removed through the abdominal incision but showed no narrowing of the bodies or of the intervertebral disks. The bone marrow was grayish red and of spongy consistency.

A lymph node measuring 2 by 1 cm. was removed from the patient's inguinal region Oct. 11, 1940. Microscopic examination showed the presence of innumerable, closely set enlarged lymph follicles. Some of the follicles were made up almost exclusively of well nucleated cells of the large lymphocytic type and embryonal hypochromatic or shadow cells. In others the follicular boundaries were lost, and although the follicles tended to retain their shape, they were enlarged and composed practically exclusively of embryonal large hypochromatic or shadow cells. On November 17 biopsy of an enlarged axillary lymph node revealed excessive numbers of fairly sharply defined large and small lymph follicles, some of which were composed of moderately chromatic cells of the large lymphocytic type and occasional embryonal large hypochromatic or shadow cells. Other sections from the same node showed recognizable follicular remnants which stained lightly and stood out sharply in contrast to scattered collections of fairly richly chromatic cells of the large lymphocytic type. The lighter staining follicular remains were made up almost entirely of large embryonal hypochromatic or shadow cells, and the node was flooded with cells of the same histologic appearance.

Microscopic examination of enlarged nodes removed from various parts of the body at necropsy showed complete replacement by loosely arranged cells of the large lymphocytic type with deeply staining rounded or oval nuclei and an occasional semilunar-shaped cytoplasm and a sparse sprinkling of large embryonal hypochromatic or shadow cells. The former cells closely resembled the well nucleated large cells of the large lymphocytic type found in the follicles and in the parenchyma of the nodes removed from the inguinal and axillary regions during life, while the latter cells were indistinguishable histologically from the large embryonal hypochromatic or shadow cells (fig. 9).

The spleen showed fairly large numbers of markedly atrophic lymph follicles. Many of them consisted of small collections of lymphocytes and were recognizable as follicles only because they were distributed around or in the immediate vicinity of the central arteries. The substance of the spleen, on the contrary, was infiltrated by richly nucleated embryonal cells of the large lymphocytic type. Among these cells was a general sprinkling of large embryonal hypochromatic or shadow cells and transitional forms of the shadow cell type.

This case is remarkable in that the enlarged inguinal nodes removed in October 1940 showed some lymph follicles composed of cells of the large lymphocytic type and others composed of large embryonal hypo-

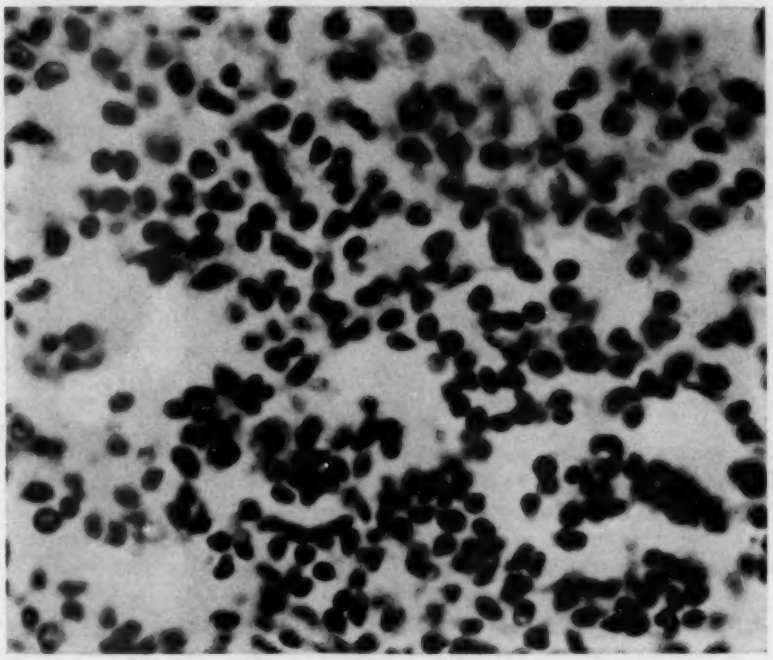

Fig. 9 (case 5).—Photomicrograph showing infiltration of a retroperitoneal lymph node by embryonal cells of the large lymphocytic type and smaller numbers of embryonal hypochromatic or shadow cells. This case is one of giant follicular lymphadenopathy undergoing transformation into polymorphous cell follicular sarcoma and thence into lymphoid leukemia of the embryonal large lymphocytic type. Hematoxylin and eosin; paraffin; × 736.

chromatic or shadow cells. Axillary lymph nodes removed a month later showed much the same microscopic picture plus invasion of the interfollicular tissues by hordes of large embryonal hypochromatic or shadow cells, indicating transformation into polymorphous cell follicular sarcoma. During life the patient's blood revealed lymphoid leukemia with a preponderance of large lymphocytes. At necropsy the greatly

enlarged nodes in various parts of the body, including the superficial nodes, showed no follicular remnants. They were composed almost exclusively of cells of the large lymphocytic type, which appeared to be identical with cells found in some of the follicles and follicular remnants in the inguinal and axillary nodes removed during life. In many instances these cells lay in clumps of syncytium. That there was a relationship between the changes in the substance of the spleen and in the lymph follicles of the enlarged superficial nodes and the development of leukemia of the large lymphocytic type is indicated by the fact that among the large lymphocytes found in the spleen and in the leukemic lymph nodes there were also numbers of large embryonal hypochromatic or shadow cells identical with those in the follicles of the superficial nodes.

From the histologic findings I am led to the conclusion that this case is one of giant follicular lymphadenopathy of the superficial lymph nodes undergoing transformation into polymorphous cell follicular sarcoma and thence into leukemia of the embryonal large lymphocytic type.

In this case there was an additional feature worthy of note in that the ribs, the sternum and vertebrae showed an extraordinary degree of osteoporosis, the interlamellar spaces being distended by polymorphous cell follicular sarcoma and the lamellas atrophied from pressure. This case again emphasizes the extent to which polymorphous cell sarcoma of lymph node origin may bring about invasion of the marrow and changes in bone (see case 3).

CASE 6.—A Russian woman aged 48, a bookkeeper, was admitted to Bellevue Hospital Nov. 24, 1937 and again May 3, 1938. On both occasions she complained of tarry stools, which had been present "off and on" for a period of several years, and occasional vomiting of large quantities of bright red blood. She admitted that she had contracted a chancre in 1935, for which she received desultory arsenical treatment over a period of about one year. The Wassermann reaction was 4 plus. The spleen was firm and enlarged, extending about 10 cm. below the costal margin on the left side. The liver was not enlarged to palpation but on percussion seemed to extend about 3 cm. below the costal margin in the midclavicular line. There were no signs of enlargement of any of the superficial nodes. The patient presented indications of a slowly progressive but fairly severe secondary anemia, the blood count at the time of admission revealing 2,000,000 red cells and 5.6 Gm. of hemoglobin.

Splenectomy (Dr. Frank B. Berry) was performed Jan. 18, 1941. The spleen weighed approximately 800 Gm. and measured 15 by 8 by 5 cm. On section it was firm, dark blue and friable. Scattered through it were huge numbers of whitish specks (fig. 10). At the time of operation Dr. Berry palpated and inspected the liver and found it to be apparently of average size and presenting a smooth surface. On exploration of the abdomen no enlarged lymph nodes were detected. A small portion of the liver was removed for biopsy.

Microscopic examination of the spleen showed vast numbers of hyperplastic lymph follicles. Many of them were sharply delimited by a zone of closely

packed small lymphocytes surrounding loosely arranged embryonal hyperchromatic cells of the large lymphocytic type and an occasional embryonal hypochromatic shadow cell. Other follicles were composed practically exclusively of cells of the large lymphocytic type, without the familiar border of small lymphocytes. The rest of the spleen together with tissue removed from the liver for biopsy showed nothing of interest in the present connection.

Decker and Little [8] published a similar case. Splenectomy was done in April 1927 on a woman 28 years of age. The spleen weighed 3,500 Gm. and measured 22.5 by 11.3 by 6.5 cm. There was no enlargement of the superficial lymph nodes. On microscopic examination the spleen was found to be riddled by hyperplastic lymph follicles. At the time of operation two enlarged nodes were found in the gastrosplenic

Fig. 10 (case 6).—Photograph of a spleen showing innumerable hyperplastic lymph follicles. The organ was removed surgically and probably represents a variety of giant follicular lymphadenopathy arising in the spleen.

omentum, the larger measuring 2 cm. in its longest diameter. Biopsy of this lymph node showed changes essentially the same as those in the spleen. Two years and five months later the patient showed no detectable signs of lymph node enlargement in any part of the body.

Whether there is a form of giant follicular lymphadenopathy which arises primarily in the spleen has not been determined. The question cannot be put at rest until necropsy reveals an enlarged spleen in which the lymph follicles show numerical and dimensional hyperplasia unassociated with any extensive enlargement of lymph nodes in other parts of the body due to follicular hyperplasia. In the case of splenomegaly

8. Decker, H. R., and Little, H. G.: J. A M A **105**:932, 1935.

recorded here these conditions were not fulfilled. It was impracticable for the surgeon to conduct other than a cursory examination of the contents of the abdomen, and of course no examination of the thoracic contents was possible. Only eight months have passed since splenectomy was done, and the patient's future is not predictable.

Whether in cases of the sort just described widespread enlargement of lymph nodes due to the same cause may arise later in life is impossible to say, but that such a contingency may arise is not beyond reasonable expectation. However, I think it is safe to assume that splenomegaly was the preponderating lesion in the 2 cases mentioned and not too bold a venture to guess that it was primary in both of them.

For a good many years an almost exactly similar doubt was current in respect to primary Hodgkin's disease of the spleen. It is now generally accepted that a marked degree of splenomegaly may be present in Hodgkin's disease before the appearance of detectable enlargement of the superficial or other groups of lymph nodes. Conversely, it is generally accepted that in Hodgkin's disease predominant if not primary lesions may occur in the abdominal lymph nodes and in the thymus or its remains without enlargement of the spleen and with or without enlargement of the superficial nodes. If in these circumstances the spleen participates in the pathologic process at all, it is apt to do so to a limited extent only.

In August 1937, Dr. Chester R. Brown, pathologist at Lincoln Hospital, permitted me to examine sections of a spleen which had been removed from a woman 37 years of age. The organ weighed 2,000 Gm. and measured 30 by 22 by 15 cm. On section the red pulp was almost completely replaced by rounded or oval whitish follicles measuring from 2 to 5 mm. in diameter. On microscopic examination by far the greater part of the follicles was made up of moderately well nucleated embryonal cells of the large lymphocytic type with, however, a sprinkling of large hypochromatic or shadow forms—polymorphous cell sarcoma. No germinal areas were observed. A lymph node removed at operation showed essentially the same changes. At the time of operation great numbers of enlarged nodes were palpated in the mesenteric and retroperitoneal regions. In October 1939 the patient died in the Montefiore Hospital, and necropsy revealed widespread polymorphous cell sarcoma of the lymph nodes superimposed on Hodgkin's disease with involvement of the liver and of many of the abdominal lymph nodes. In some of the abdominal nodes the changes of Hodgkin's disease and of polymorphous cell sarcoma could be seen in the same hyperplastic follicle. The two diseases in the same follicle revealed no evidence by means of which they could be traced one into the other. They appeared as independent histologic alterations except for the fact that the cells at the point of meeting intermingled for a short distance, retaining at the

same time their individual structural characteristics. In the liver there was a combination of multiple large collections of lymphocytes with numbers of germinal areas in them, the customary signs of polymorphous cell sarcoma and the characteristic changes of Hodgkin's disease—three different, although closely related, disease processes in the same organ. The lymphocytic collections with germinal areas in them appeared to me to represent hyperplastic depots belonging to the auxiliary lymphoid system.

PARALLELISMS BETWEEN HODGKIN'S DISEASE AND GIANT FOLLICULAR LYMPHADENOPATHY

There are several parallelisms between giant follicular lymphadenopathy and Hodgkin's disease. Clinically the two diseases are easily confused because in both the first detectable signs of lymph node enlargement are manifested in the superficial nodes, either localized or generalized. In both diseases the enlarged superficial nodes may be distributed with almost unlimited diversification—no 2 cases of Hodgkin's disease or of giant follicular lymphadenopathy ever "look alike." Nevertheless, the clinical resemblance between the two conditions cannot be denied. However, histologic differences enable the examiner to distinguish one from the other almost at a glance.

For many years the enlarged superficial nodes in Hodgkin's disease were regarded as multiple primary foci. At the present moment the belief is universally entertained that the enlarged superficial nodes are merely outposts stationed at a distance from the main body of enlarged nodes in the deeper parts or from the larger lymphoid organs, that is to say, the thymus and the spleen.

The opinion seems to be prevalent at this moment that the enlarged superficial lymph nodes in giant follicular lymphadenopathy are multiple primary foci. On the contrary, it is shown in this paper, I believe, that giant follicular lymphadenopathy, like Hodgkin's disease, is primarily a disease of the deeper lymph nodes and that the enlarged superficial nodes are outposts situated at a distance from the main body of enlarged nodes. This conclusion is based on the fact that in giant follicular lymphadenopathy as well as in its immediate derivative, polymorphous cell sarcoma, the abdominal lymph nodes, or the abdominal and thoracic nodes combined, are enlarged out of all proportion to the superficial nodes, which are comparatively small and few. It is also shown in this paper that there are grounds for the belief that there is a form of primary giant follicular lymphadenopathy of the spleen. Unlike Hodgkin's disease, however, giant follicular lymphadenopathy has not thus far been described as a primary disease of the thymus. Although lymph follicles occur in the medulla of the human thymus, they are rare,[9]

9. Wegelin, C.: Centralbl. f. allg. Path. u. path. Anat. **29**:441, 1918.

and perhaps it is not overpresumptuous to predict that giant follicular lymphadenopathy may eventually be described as a primary disease of the thymus.

The most significant parallelism between Hodgkin's disease and giant follicular lymphadenopathy is that in both diseases the earliest observable change in the node is hyperplasia of the lymph follicle. However, the hyperplastic follicle soon disappears and is almost always replaced by overgrowth of lymphoid cells; only occasionally is the follicle or its remnants retained in the histologic panorama of developing Hodgkin's disease. Longcope described hyperplastic follicles and follicular remnants in the nodes from patients with Hodgkin's disease six years before I observed them. His paper, which attracted well deserved attention, was largely descriptive. To his description I would add the conclusion, based on my own experience, that the hyperplastic lymph follicle is a prerequisite for the development of Hodgkin's disease. If this is true, it establishes not only another and important resemblance between Hodgkin's disease and giant follicular lymphadenopathy but a fundamental relationship between them. This conclusion is further supported by the fact that signs of giant follicular lymphadenopathy, polymorphous cell sarcoma and Hodgkin's disease sometimes exist side by side in the same lymph node.

Another parallelism between Hodgkin's disease and giant follicular lymphadenopathy is to be found in the fact that spontaneous diminution in size or even complete disappearance of the enlarged lymph nodes may occur in both diseases and that both are almost certain to recur sooner or later.

In addition to the parallelisms outlined, giant follicular lymphadenopathy and Hodgkin's disease display certain microscopic peculiarities of development which I purpose to outline in some detail but for which I know no appropriate covering name. For example, giant follicular lymphadenopathy and Hodgkin's disease are primarily disturbances of the lymphoid system. In both diseases the initial change consists in the appearance of aggregate bodies in the form of hyperplastic lymph follicles. In giant follicular lymphadenopathy the hyperplastic follicles practically without exception maintain their aggregate structure from beginning to end (fig. 4, case 2), while in Hodgkin's disease the hyperplastic follicles, with rare exceptions, disappear either in whole or in part early in the process of development and are replaced by diffusely distributed lymphocytes. In giant follicular lympadenopathy eosinophils are seldom present in the peripheral blood either in normal or in abnormal numbers. In the hyperplastic lymph nodes I have seen them in small numbers once or twice only; in a single case they were present in abundance. This would seem to indicate no noteworthy increase in the activity of the bone marrow. In Hodgkin's disease, on the other hand, eosinophils are fre-

quently present in the peripheral circulation in normal numbers and sometimes in high abnormal numbers. They have often been recorded as constituting as much as 60 per cent of the total number of leukocytes in the peripheral blood. As to the lymph nodes, the eosinophils are detectable in a large percentage of all routine microscopic preparations and are often found in association with eosinophilic myelocytes. These findings are indicative of a degree of activity in the bone marrow noticeably transcending the normal.

In giant follicular lymphadenopathy the richly nucleated, unusually large immature cells of the lymphocytic type may proliferate in such numbers as to bring about generalized enlargement of the lymph nodes—a form of lymph node sarcoma (case 4). In giant follicular lymphadenopathy proliferation of the immature cells of the large lymphocytic type may also bring about enormous enlargement of both the superficial and the deep lymph nodes in association with a large cell type of lymphoid leukemia (case 5). In Hodgkin's disease, on the contrary, the involved tissues may show variable numbers of lymphocytes of either the large or the small cell variety or both, but leukemic transformation has not been shown to occur nor has it been shown that these cells develop into lymphosarcoma. However, in Hodgkin's disease the presence in the involved tissues of megalokaryocytes is constant, and without them, in my experience, the diagnosis of Hodgkin's disease cannot be made with assurance. Their presence in extramedullary tissues is obviously an indication of hyperactivity of the bone marrow and may be logically construed as a sign of transplantation of cells by the process of embolism, the emboli being filtered out by the hyperplastic lymph nodes, whose function is that of a filter.

In other words, both giant follicular lymphadenopathy and Hodgkin's disease are conceived in hyperplasia of lymph follicles. Giant follicular lymphadenopathy exhibits a persistent tendency to adhere to the pattern set by the cells of the hyperplastic follicles. Hodgkin's disease soon departs from this pattern and emerges as a disease in which the characteristic cells are of the type produced exclusively by the bone marrow.

SUMMARY

Giant follicular lymphadenopathy is oftenest a primary disease of the lymph nodes of the abdomen or of the abdomen and the thorax combined. Whether the disease may arise in the spleen has not been fully determined. It may persist as an independent disease, or it may undergo transformation into polymorphous cell sarcoma. In either event enlarged lymph nodes arise over a wide geographic expanse with or without splenomegaly, and the picture thus produced is almost always mistaken clinically for Hodgkin's disease. The histologic changes in giant follicular lymphadenopathy alone or in its immediate derivative, polymorphous

cell sarcoma, are entirely different from those of Hodgkin's disease. The distinction between them can be made with certainty only on biopsy of diseased nodes. That the differential diagnosis is of importance is signified by the fact that in a noteworthy percentage of all cases, generalized giant follicular lymphadenopathy is readily susceptible to roentgen therapy in small doses and that polymorphous cell sarcoma arising from the hyperplastic lymph follicles of giant follicular lymphadenopathy is more resistant to such therapy but distinctly less resistant than Hodgkin's disease, which is apt to be extremely refractory.

Giant follicular lymphadenopathy in its earlier stages is a comparatively innocent disease. However, as the lymph nodes increase in size and number, they interfere with the venous return, and various grades of serous transudation ensue, including general anasarca. Polymorphous cell follicular sarcoma may likewise pursue a comparatively innocuous course for a while, but in its later stages it may also cause serous transudation and, in addition, bring about widespread destruction of tissues beyond the lymphoid system. Thus, as has been shown in this paper, it is capable of invading bone in such fashion as to prepare the way for spontaneous fractures (fig. 7, case 3). Hodgkin's disease may pursue an almost exactly similar course.

Giant follicular lympadenopathy may undergo transformation into a form of generalized sarcoma of lymph nodes in which the cells are very large and immature and represent, apparently, younger forms of cells of the embryonal large lymphocytic type referred to throughout this paper (fig. 8). Likewise, giant follicular lymphadenopathy may undergo transformation into a variety of leukemia characterized by generalized enlargement of lymph nodes in which the predominating cells are of the embryonal large lymphocytic type but among which there is a more or less liberal sprinkling of embryonal hypochromatic or shadow cells (fig. 9, case 5). The latter have not thus far been identified in the peripheral circulation.

Many of the observations embodied in this paper support the view that there is a series of diseases of the lymphoid system, commencing with giant follicular lymphadenopathy, each member of which is conceived in hyperplasia of lymph follicles—in short, that in all of them hyperplasia of the lymph follicle is a prerequisite for development. This series includes giant follicular lymphadenopathy, Hodgkin's disease, certain forms of lymphoid leukemia, lymphosarcoma, the immature large cell sarcoma of lymph nodes described in this paper and probably others. All of them, except, of course, giant follicular lymphadenopathy, appear in the end as divergent histologic changes, although initiated, it would seem, by the same cause.

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KAPOSI'S SARCOMA

A CRITICAL SURVEY

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In 1872 Kaposi described the lesion now generally called Kaposi's sarcoma and named it idiopathic multiple pigment sarcoma. In 1894 he changed the designation to sarcoma idiopathicum multiplex haemorrhagicum. Since then numerous investigators have attempted to classify it according to their views on its cell origin, so that various names have been suggested.

It is a disease of geographic rather than racial incidence, affecting principally the inhabitants of central and southeastern Europe and their descendants. It is common in Russia, northern Italy, Poland and those countries bordering the Mediterranean and Caspian seas. It is uncommon in Negroes. It has its highest incidence in persons in the fifth to the seventh decades of life and is rare in children, less than 1 per cent of the reported cases occurring under the age of 10 years. It is about twenty times more common in males than in females. It occurs chiefly in laborers and outdoor workers.

The disease is most commonly primary in the skin, though the primary process has been reported in almost all parts of the body. It was the cutaneous type which Kaposi described. The lesion usually begins as a reddened macular area on an extremity, most commonly a lower extremity. The edges are usually definite but there is no characteristic shape. The macule varies greatly in size, depending on how long it has been present. As the lesion progresses, the color of the involved area darkens, becoming first dusky red, then bluish and finally brown or black. This change of color is understandable after microscopic study of the lesion. At first there is an increase in the supply of blood to the part through the cavernous vessels. At this time the area is livid. But as the cellular elements of the tumor increase, there is stagnation of the blood, and finally hemorrhage with rather remarkable pigmentation, which probably results from the disintegration of the blood. The involved area soon becomes elevated and in the early

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stages resembles a varicosity. As the tumor ages, becoming more cellular and less vascular, it becomes firm, and its appearance is that of a true neoplasm. The lesion rarely regresses spontaneously, although it may remain stationary for months. Healthy areas of skin proximal to the lesion may soon become involved. The older lesion may fade out, become keratinized and present a scaly surface. The subcutaneous tissues become infiltrated, and the lymphatics are frequently blocked, causing an immense edema, even elephantiasis, of an extremity. Sometimes the lesions are pruritic, and trauma may cause them to bleed freely. Sooner or later, lesions usually appear in the viscera. Some writers believe these to be spontaneous multiple primary growths because they are able to find all the stages of development in these lesions. If we accept the opinion that this disease is cancer from the outset, and such is our premise, we may regard these secondary lesions as metastases, following the usual course of cancer. Death may occur from infiltration of the lungs, the liver or the spleen but by far the most common sequence is metastasis to the intestinal mucosa with ulceration causing hemorrhage and exsanguination. When the tumor is primary in the viscera, it is usually not found in the skin. Choisser and Ramsey [1] reported 2 cases with the lesion primary in the right atrium of the heart. We have autopsied 1 case of the same type. All 3 of the patients died of hemorrhagic tamponade secondary to ulceration of the tumor. Metastasis to lymph nodes is not general, but it occurs, and the metastatic lesions reproduce the primary cellular picture.

If sections are made of the very early lesions in the skin, the microscopic picture is that of cavernous hemangioma. The sinuses vary in size and shape, they contain blood and they are lined by normal-appearing endothelial cells. No perithelial cells could be distinguished in any of our slides. As a lesion progresses, it is seen to penetrate into the surrounding normal tissues, splitting muscle bundles and infiltrating the fat. This infiltration is characteristic of cancer, and this characteristic is seen in the very early lesions. The sinuses are less perfectly formed at this stage, and they are surrounded by masses of fusiform cells without pattern, the nuclei of which are indistinguishable from those of the endothelial lining cells. A little later the sinuses become indistinct and are represented by spaces which are filled with blood. There are hemorrhage and considerable red cell degeneration, as shown by the quantities of pigment which take the iron stain. The endothelial lining is now almost indistinguishable, and there is an apparent transition of the endothelial into the fusiform cells. The latter cells proliferate to form masses, some of which are quite avascular. Sections through these

1. Choisser, R. M., and Ramsey, E. M.: South. M. J. **33**:392, 1940; Am. J. Path. **15**:155, 1939.

areas are easily confused with fibrosarcoma or even neurogenic sarcoma. These solid masses occupy the corium, pushing upward to flatten the papillae, causing thinning of the epidermis. It is these masses that give rise to the nodular gross appearance. By this time there is usually an inflammatory reaction, as shown by an infiltration of lymphocytes, plasma cells and a few macrophages. This may be accounted for by the hem-

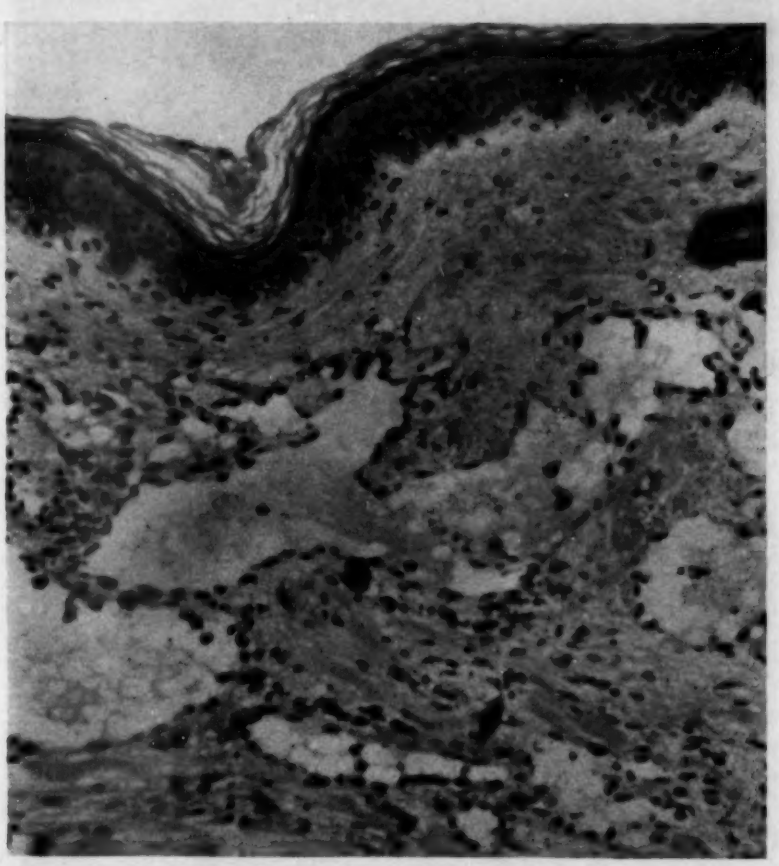

Fig. 1.—Photomicrograph of a section of skin. This is a very early lesion, showing the cavernous sinuses lined by endothelium.

orrhages. Differential stains give the staining reaction of young fibrous tissues. In some tumors these cells show definite malignant characteristics. In some instances the solid cell masses have the appearance of endothelioma. The cells are polyhedral and arranged in sheets about irregular sinuses, some of which contain erythrocytes.

The duration of the disease varies from eight months to twenty-five years although in the majority of instances the process terminates

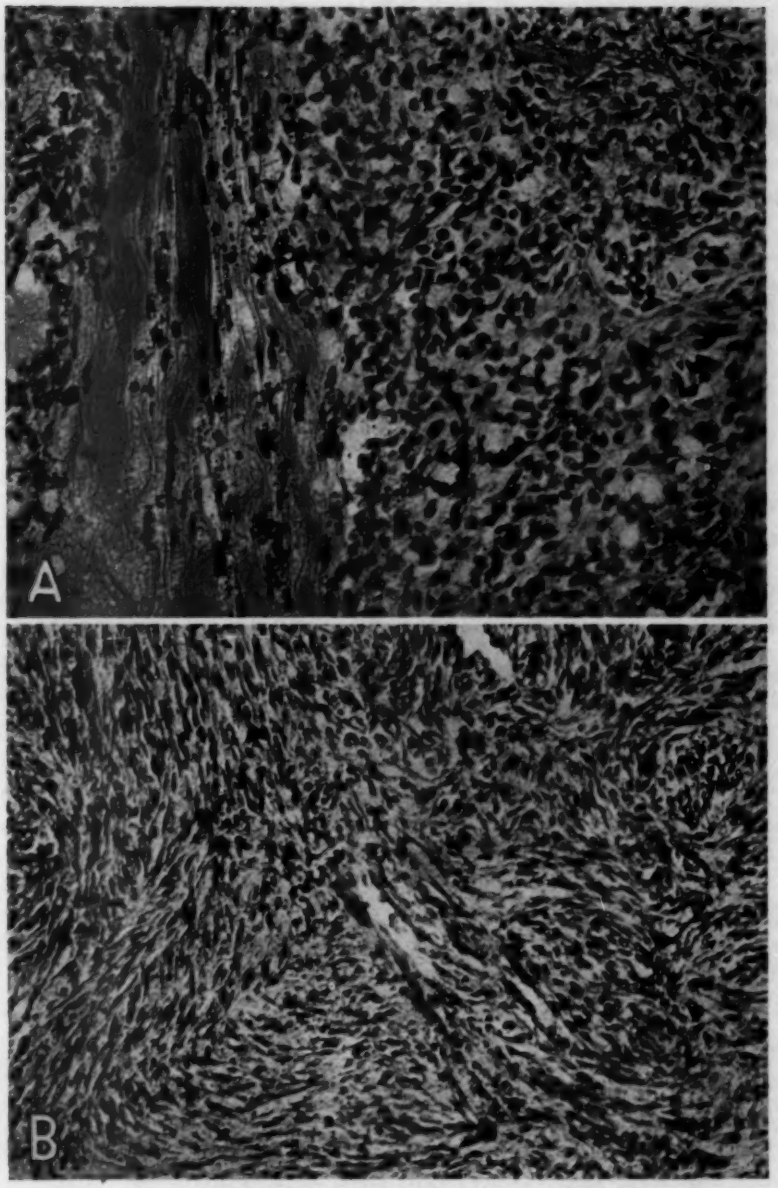

Fig. 2.—*A*, photomicrograph of a section taken from a subcutaneous area. This is an older lesion than that shown in figure 1. Now there is only a suggestion of sinus formation. There is proliferation of the endothelial lining cells, and some of them show spindling. *B*, photomicrograph of a well developed lesion. There is almost no sinus formation. Practically all of the endothelial cells have undergone spindling, so that the tumor resembles neurofibroma or fibrosarcoma.

after five to ten years. In a few cases it has been reported to have regressed, with the patient remaining free of symptoms for a number of years. We are tempted to question the diagnosis in these cases since by far in the majority of over 600 reported cases the disease was progressive, though sometimes remittent in character.

Roentgen therapy probably gives the best results in the treatment of this disease. The early lesions may respond to small doses of low voltage, but they almost invariably recur, and the secondary tumors are more resistant to treatment. Solution of potassium arsenate U. S. P.

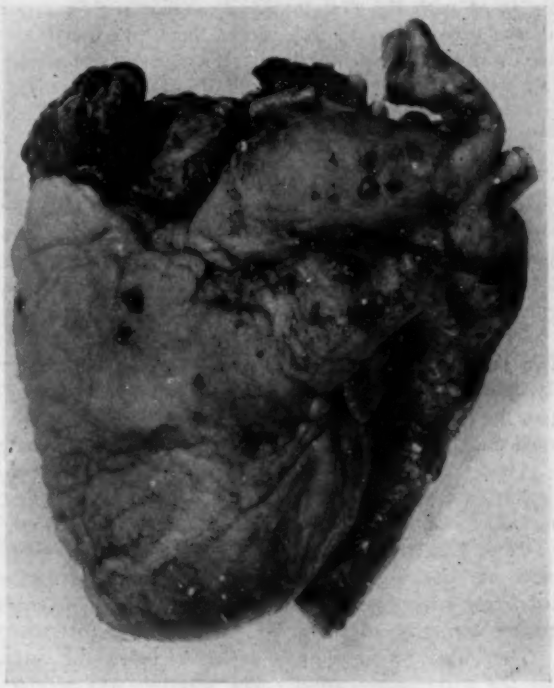

Fig. 3.—Anterior aspect of the heart showing the tumor arising from the right atrium and spreading over the epicardial surface.

by mouth or solution of sodium arsenate N. F. by injection is said to produce some temporary good results.

REPORT OF CASES

Case 1.—A white woman aged 46 years, American born, was admitted with chief complaints of pain and swelling in her right leg. Her history revealed that after menarche at 11 years of age she noted small areas of subcutaneous hemorrhage above the right knee. She was operated on for this condition, without subsequent improvement. The leg remained about three times the normal size after the operative procedure, though the swelling would subside on elevation.

About six years before her death, her second toe became inflamed and discolored, necessitating amputation. In the last two years of life the disease progressed rapidly, the lesions becoming confluent until they involved the entire leg. A second attempt to improve the condition by surgical measures resulted in an infection, and the patient died of sepsis eleven days after operation. A piece of tissue was taken for microscopic study, but permission for autopsy was refused.

Case 2.—A white man aged 63 years, Italian, had noticed, three years before he was studied at Temple University Hospital, a small nodule on the outer aspect of the dorsum of the left foot. It was about 1 cm. in diameter and rather firm but not painful. This nodule grew slowly but progressively. A few months later a similar nodule appeared posteriorly under the lateral malleolus. It grew rapidly and apparently fused with the first mass. A similar nodule appeared later over the dorsum of the proximal phalanges of the third and fourth toes. There was no pain. There was no history of injury. Roentgenograms showed a soft tissue tumor invading bone. Biopsy led to a diagnosis of Kaposi's sarcoma, and amputation of the foot was successfully done. The patient was discharged in good condition.

Case 3.—A white man aged 46 years, Jewish, complained chiefly of weakness and fatigue. He had been well until two and a half years before admission to the hospital, when several small scaling skin lesions developed on the foot and leg. After administration of serum for pneumonia, deep purple patches developed on his left hand, knee and ankle. His hand showed blue thickened areas in the palm and the region of the index finger. His right leg became edematous from the toes to the lower part of the abdomen. Numerous small hemorrhagic areas developed over the whole leg. He was given roentgen therapy, but the progress of the disease was not noticeably altered. He died, and a complete autopsy was done.

Post mortem the lower extremities and the lower one third of the trunk showed a remarkable edema. The feet, ankles and legs were approximately twice their normal size. There was a tumor process involving the right foot, ankle, leg and thigh, the left foot and ankle, both lateral aspects of the chest and abdomen and almost the entire back. The lesions had definite margins, were dark blue and measured from 0.5 to 3 cm. in diameter. Some were macular, while others rose above the skin as much as 1.5 cm. The latter had the appearance of cavernous hemangioma and bled freely when incised. Some of the lesions were covered with thick dark scales resembling those of psoriasis. On the right foot there were several healed areas. They were round, slightly depressed below the surface of the skin and paler than the surrounding areas. The scrotum was solidly involved with tumor. The skin had a leathery consistency when incised. The liver contained a tumor measuring 1.5 cm. in diameter, diagnosed as cavernoma. The serosal surface of the distal two thirds of the ileum revealed encircling hemorrhagic bands, from 1 to 5 cm. wide, in which the wall was greatly thickened. On the mucosal lining in these areas there were many deep purple firm papillary projections and plaques. The lumen of the bowel was filled with partially digested blood.

Case 4.—A Negro aged 60 years had been well until one year before his admission, when he began to suffer attacks of paroxysmal dyspnea, which often awakened him from sleep. His heart was found to be moderately enlarged to the left, and there was a blowing systolic murmur at the apex, transmitted to the axilla. The liver was enlarged. An electrocardiogram suggested coronary disease. The patient died suddenly, and a complete autopsy was done.

The pericardial sac was immensely dilated, containing over a liter of fresh, unclotted blood. The heart showed moderate concentric hypertrophy. Arising

from the wall of the right antrum just distal to and to the right of the base of the right auricle there was a tumor mass measuring 4 cm. at its base. It varied in color from a dark purple to a cyanotic red. It was nodular and hemorrhagic. Just distal to and to the right of this region a somewhat smaller tumor arose from the right atrial wall and extended into the atrial cavity for a distance of 1.5 cm. Scattered over the remaining portion of the atrial wall down to the anterior surface of both the right and the left side of the heart and up over the conus arteriosus and the base of the aorta there were numerous plaques of tumor tissue. One mediastinal lymph node revealed metastasis.

COMMENT

Most of the early writers who described the lesions of Kaposi's disease were uncertain as to whether this process was of an inflammatory or a neoplastic origin. Their conception of the earlier lesions made it appear to be inflammatory in nature. They saw hemorrhage, pigmentation, infiltration by lymphocytes and plasma cells and proliferation of cells which were not unlike those in chronic granulation tissue. It is not surprising, therefore, that for many years the disease was thought to be a chronic low grade inflammatory process. The later stages of the disease, however, are typically neoplastic. Since the disease progresses so slowly, it is probable that different men saw the same lesion at varying stages of its development, some believing it to be of an inflammatory nature, others regarding it as a tumor or a tumor implanted on an inflammatory base. Most writers now feel that the process is a neoplasm of low grade malignancy from its beginning.

If the premise of neoplasia is accepted, the question of cell genesis immediately arises. The histologic picture is complex, the vascular channels predominating in some areas, fibrous-like tissue in others and endothelial hyperplasia in still others. It is difficult, therefore, to decide where the tumor belongs in a cell type classification. Choisser and Ramsey [1] circumvented the difficulty of the multiple cell types by suggesting that all the cells of the various types might arise from the reticuloendothelial system. They therefore suggested the name "angioreticuloendothelioma." That is doubtless true, but their conception associates the tumor with the reticulum cell sarcoma or with the vascular endothelioma rather than with the basically angioid tumors with which we feel it belongs. In view of the fact that the fibrous wall of a vessel and its endothelial lining are closely associated in their embryologic origin it does not seem too radical to suggest that there may be a transition between the fibroblasts and the endothelioblasts. We are not the first to make this suggestion. If we can depend on cellular character as a reliable means of tumor classification, this is actually what appears to take place. Dörffel [2] in his detailed histologic report described the endothelial cells which originally line the sinuses breaking away to form solid masses

2. Dörffel, J.: Arch. Dermat. & Syph. **26**:608, 1932.

and finally becoming spindled to resemble fibroblasts. Our tumors showed the same apparent transition. The cell shape changes, but the nuclear characteristics remain identical. If, as some writers think, the endothelial lining of the normal blood vessel is the specialized product of its fibrous wall, or, perhaps better, if the fibrous wall is the product of the primitive endothelial vascular tube, it seems sensible to regard the Kaposi sarcoma as a cancer. If one accepts the autonomous neoplastic reaction of the supportive connective tissue of the nerve in the development of the characteristic tumor, the neurofibroma, why is it not as logical, by analogy, to conceive of a similar reaction in the supportive tissues of the vessel wall in an effort to understand the genesis of Kaposi sarcoma?

It is with considerable trepidation that we attempt to classify the protean tumors which apparently spring from the blood vascular system. The very purpose of classification is to simplify, and the complexities of these lesions defy a simple grouping comparable with that of other tumors. Many trustworthy workers have written on various lesions in this group as separate entities until a vast heterogeneous literature has accumulated, but many fail to comprehend the subject because of failure to correlate the facts concerning each separate disease. We therefore present the following classification in an attempt to consider these conditions as related phenomena.

Classification

A. Non-neoplastic vascular lesions
   1. Varicosity
   2. Granulation tissue
   3. Telangiectasia
   4. Congenital arteriovenous fistula

B. Noncancerous neoplasia of blood vessels
   1. Angioma { Hemangioma / Lymphangioma
      (*a*) Cavernous
      (*b*) Capillary (angioblastoma?)

C. Cancer of blood vessels
   1. Congenital angioblastoma (metastasizing angioma)
   2. Angiosarcoma (Kaposi's disease)
   3. Endothelioma

D. Mixed neoplasia involving blood vessels
   1. Glomus tumor

If we accept the usual definition of a tumor as a purposeless, autonomous growth of tissue, we must cull out many of the lesions of blood vessels as non-neoplastic. Of these the common varicosity is certainly not a tumor, since mechanical stasis is the usual etiologic agent.

The vascular elements in chronic granulation tissue may become so prominent as to suggest tumor proliferation, yet granulation tissue is a purposeful growth which, if successful, culminates in a healed scar. Sometimes this process may result in more or less permanent telangiectasia which is easy to confuse on microscopic study with the glomus tumor or even with angioblastoma. The arteriovenous fistula is usually congenital, and if multiple, as it frequently is, it gives rise to characteristic symptoms referable to the heart. This anastomosis may be a simple, single channel or it may form a plexiform mass of tortuously convoluted tubules simulating cavernous hemangioma.

The remaining lesions, those which are commonly designated as angioma, angioblastoma, vascular endothelioma and Kaposi's sarcoma, satisfy the criteria of the definition of neoplasia and should be considered under the heading of true tumors. Of these, the most common are typically benign. Thus angioma is a nonprogressive or slowly progressive mass of tissue which compares with the benign, frequently multiple tumor of the fibrous supportive tissue of the peripheral nervous system, the neurofibroma. Tumors diagnosed as angioma may be classified according to their genesis from blood or from lymph vessels. That these tumors are usually congenital is suggested by the preponderance of their incidence in early childhood. These tumors have been reported in practically every tissue in the body, being commonest in the skin and the liver but found not infrequently in the muscles, the spleen, the bones and the gastrointestinal tract. They are sometimes cystic. Lindau described them in the region of the brain stem and found these frequently accompanied by similar lesions in the viscera. Von Hippel [3] described angioma involving the retina. Both clinically and histologically these tumors may be divided into the cavernous and the capillary type. In most cases of neoplasm one assumes that the more adult the cell type the less malignant the tumor is apt to be. In the case of the common cavernous angioma the unit is the blood vessel. These vessels are well formed, and the tissue is certainly morphologically adult. It is interesting to note that the tumors that are wholly cavernous in type are probably always benign and are the easiest to treat successfully. In the capillary type, however, the vessels are less well developed, there is more endothelial proliferation and in general the structure is more embryonic than in the purely cavernous type. It is the capillary type which may become cancerous, and it is this type which so often recurs after surgical or roentgen treatment. It seems possible then that the cavernous angioma is the only representative of the benign group and that the capillary angioma should be classified with angioblastoma.

Of those vascular tumors which are commonly considered cancerous there are three broad groups. The most adult of these in which the

3. von Hippel, E.: Arch. f. Ophth. **59**:83, 1904.

vessel is still the unit is represented by the slowly progressing angioblastoma, usually congenital and commonly remaining local. Sometimes, however, these tumors may suddenly metastasize widely throughout the body. When they behave in this manner, they have been called angiosarcoma. The second group, usually arising in the skin and when so doing called Kaposi's sarcoma, is certainly less adult in type and is slowly cancerous from the beginning. Here some irregular vessels are formed, but the preponderance of the tumor is made up of spindled or endothelium-like cells which grow in solid invasive masses. Because of its obvious vascular origin and its vascular neoplastic nature, we believe it should be classified as angiosarcoma. We are tempted to include under a third broad heading that large group of tumors which apparently arise from the endothelial lining of blood vessels and are commonly designated as endothelioma. In these the vessel is no longer the unit; the cells take on a completely embryonic form and become correspondingly more malignant.

There is still a heterogeneous fourth group of tumors in which the vessels are an important but not the only constituent. These may be designated as mixed vascular tumors. One of the best known of them is the glomus tumor, which contains vascular, muscular and neural elements. Those tumors classed as angioma which are associated with squamous cell hypertrophy and basal cell hyperplasia are respectively termed angiokeratoma and cylindroma. Sometimes there are angiomatous proliferations accompanying tumors of fibrous tissue origin, such as chondroma and lipoma, but these are probably the vascular response to the neoplastic stimulus of the predominant cell type.

SUMMARY

In this report we review the clinical and pathologic aspects of the disease commonly termed Kaposi's sarcoma and present 4 cases which are more or less clinically typical, in all of which there were satisfactory biopsies and in 2 of which the patients came to autopsy. One of the patients was a Negro and another a woman. In 3 cases the process originated in the skin, and in 1 of these the patient died of hemorrhage from a visceral metastasis. In the fourth case the tumor was limited to the heart. The nature of the disease is discussed, and an attempt at classification of the tumors and tumor-like lesions of the blood vascular system is made, in which the Kaposi sarcoma is placed with the vascular cancers. In conclusion, we believe that the neoplasm in question is the cancerous representative of the tumors of the blood vessels and that as such it should be called "angiosarcoma," reserving the term "Kaposi's sarcoma" for the subvariety which arises in the skin.

Temple University School of Medicine.

Case Reports

CONGESTIVE SPLENOMEGALY (BANTI'S DISEASE) DUE TO PORTAL STENOSIS WITHOUT HEPATIC CIRRHOSIS; ANEURYSMS OF THE SPLENIC ARTERY

Report of a Case with Necropsy

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Since less than 100 cases of aneurysm of the splenic artery have been reported and since we have found only 2 cases in which this unusual lesion was associated with Banti's disease, we report the following case. The aneurysms were not diagnosed clinically and had nothing to do with the patient's symptoms or death. Although the patient died of repeated gastric hemorrhages from bleeding varices, a supposedly late phenomenon of Banti's disease, there was no cirrhosis in the liver. Equally significant was the presence of stenosis of the portal vein. We believe the findings in this case provide evidence supporting the opinions stated by Thompson [1] regarding the genesis of Banti's disease. We are in accord with him in his suggestion to employ the term "mechanical congestive splenomegaly" in preference to "Banti's disease," whenever practicable.

REPORT OF CASE

A 54 year old white woman was admitted to the hospital on Feb. 8, 1941 because of weakness, tarry stools and vomiting of bright red blood. On January 28, 1941 five "bad" teeth were extracted, and on January 31 seven more were removed. On February 4 she felt very weak and called her physician, who noticed considerable pallor. He prescribed a tonic, attributing the weakness to the extraction of the teeth. Eighteen hours before her admission, tarry stools were noted, and just before admission she vomited a large amount of bright red blood. Until the onset of this illness she had enjoyed reasonably good health except that during the past few years there had been several attacks of pain in the right upper quadrant of the abdomen. These attacks were brought on by fatty foods but were never so severe as to require a physician.

The patient was a very pale, weak, somewhat obese middle-aged woman. There were slight tenderness in the gallbladder region, displacement of the cardiac impulse 2 cm. to the left and five infected tooth snags with some local gingivitis. Her temperature was 98.8 F. (37.1 C.), pulse rate 120, respiratory rate 28 and blood pressure 100 systolic and 45 diastolic. Examination of the blood showed 1,500,000 erythrocytes per cubic millimeter, 24 per cent hemoglobin (Haden Hausser) and 71 per cent polymorphonuclear leukocytes. The result of urinalysis was not remarkable. Blood chemistry tests gave values within normal limits.

After thirty-five days in the hospital, the patient died. During this time she had frequent episodes of hematemesis and melena, for which she received sixteen

From the laboratory of Trinity Lutheran Hospital.

1. Thompson, W. P.: Ann. Int. Med. **14**:255, 1940.

blood transfusions, representing 7,450 cc. of whole blood. The highest erythrocyte count was 2,500,000, and the highest hemoglobin content was 40 per cent. Most of the erythrocyte counts were less than 2,000,000, and most of the hemoglobin determinations were around 30 per cent. Five days before death a hemoglobin content of 20 per cent was reported. Except on one occasion, all the leukocyte counts were under 10,000. The differential counts averaged about 70 per cent polymorphonuclear leukocytes. Her temperature ranged between 100 and 102 F.

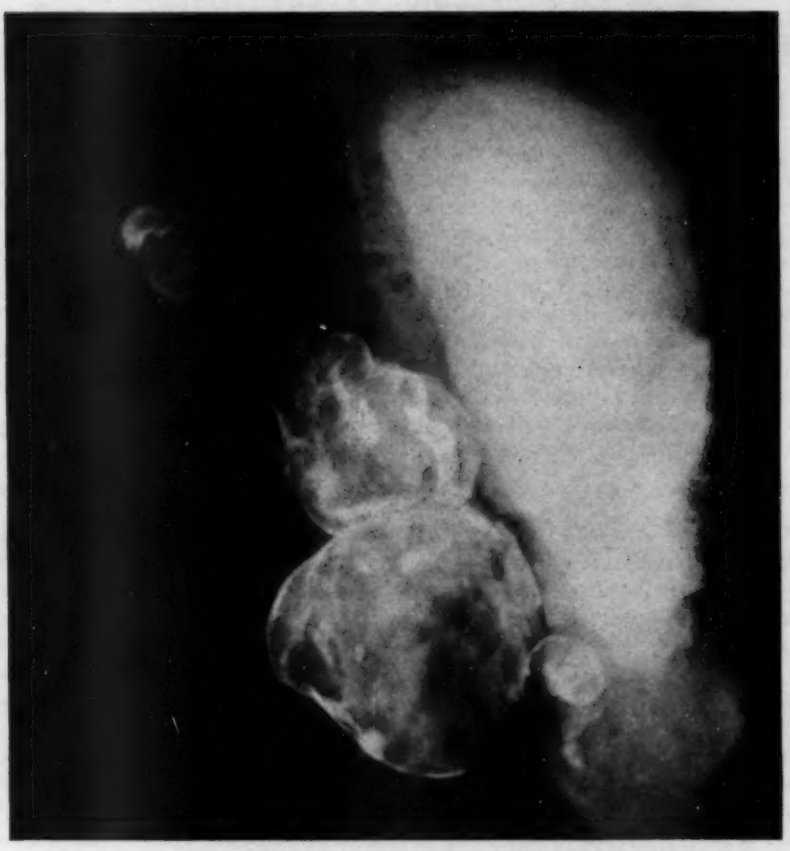

Fig. 1.—Roentgenogram of the aneurysms and spleen.

(37.7 and 38.8 C.). Her condition was so critical that at no time during her illness was the making of roentgenograms thought advisable; neither was she ever in condition for a surgical procedure.

The clinical picture, then, was that of a middle-aged woman, previously well, suffering from sudden onset of severe hematemesis and melena of unknown cause. Features of possible significance in the history were the recent extraction of a number of "bad" teeth and episodes suggestive of recurrent cholecystitis. The clinical diagnoses were (1) bleeding esophageal varices of uncertain cause, possibly due to Banti's disease, or (2) peptic ulcer, or (3) gastric cancer.

Necropsy.—Inspection revealed a well developed, rather emaciated middle-aged woman. She appeared to have been obese at one time. There was pitting edema of the lower extremities to a point several centimeters above the knees. The abdomen was flat. Decubital ulcers were present over the bony prominences posteriorly.

The head was not opened.

The left pleural cavity contained approximately 1,000 cc. of thin straw-colored fluid. The right pleural cavity contained approximately 800 cc. of a similar fluid. Both lungs were edematous and showed moderate basal atelectasis posteriorly. There was rather intense bilateral hypostatic congestion with possibly early bronchopneumonia. Approximately 70 cc. of straw-colored fluid was found in the pericardial sac. The heart was slightly enlarged and pale, and there was some serous atrophy of the epicardial fat.

The peritoneal cavity in general was free from significant changes and contained approximately 400 cc. of thin straw-colored fluid. The gastrointestinal tract as a whole was not remarkable. The stomach was small and contracted, and contained no blood. The liver was slightly smaller than normal and had a smooth capsule. Cut sections of the liver showed some passive congestion. It cut easily, with no apparent increase in fibrous tissue. The gallbladder was slightly dilated and filled with thick green bile. Its walls were slightly thickened, but the mucosa was smooth and no stones were found. The spleen was firmly held in place under the left leaf of the diaphragm by a great many old, dense fibrous adhesions. Exploration revealed some calcified tumor masses adhering to the hilar surface of the spleen and adjacent structures by fibrous adhesions. Dissection showed these tumors to be saccular aneurysms of the splenic artery, all with calcified walls. The splenic artery itself was free from atheromatous change but was tortuous. Its midportion was diffusely dilated and measured 22 mm. in circumference. In this instance, the splenic artery divided into two main branches. At the point of division was an aneurysm, measuring 2.5 by 1.8 by 1.8 cm., containing postmortem clot. The superior branch of the splenic artery bore two smaller aneurysms, one measuring 1.5 by 2 by 1.5 cm. and the smaller 1.2 cm. in diameter. The lower branch of the splenic artery passed along the shell of a calcified aneurysm that measured 5.5 by 4.5 by 4 cm. This particular aneurysm was filled with old, laminated blood clot, part of which was reddish brown and part of which was pale and granular. This aneurysm adhered to a similar structure, measuring 4 by 3 by 3 cm., which was densely adherent to the hilus of the spleen. A smaller aneurysm, about 1.5 cm. in diameter, was found where the lower branch of the splenic artery entered the spleen. The spleen itself had a pale, thickened capsule with numerous fibrous perisplenic adhesions. It measured 18 cm. in length by 9 cm. in breadth. Near the middle of the convex surface of the spleen was a transverse depression. On gross section this proved to be due to a calcified subcapsular bar, measuring 0.5 cm. in diameter and 7 cm. in length. The splenic pulp was light brown and was fibrous. The malpighian bodies could not be seen, and the vessels were widely dilated. The pancreas showed no significant change. The adrenal glands were slightly enlarged but showed no other changes. Both kidneys were pale but otherwise not unusual. The pelvic organs were not remarkable. No enlarged lymph glands were discovered.

The portal vein was formed by the union of the combined superior and inferior mesenteric veins with the splenic vein at the lower border of the neck of the

pancreas. It passed through the neck of the pancreas, describing a U-shaped turn to the right. At the completion of this turn the vein emerged from the pancreas and passed directly to the liver. The caliber of the intrapancreatic portion of the portal vein was distinctly diminished. This was due to several yellow calcified thin rings located in the wall of the vein, partially encircling it. The largest of these rings was 12 mm. long and 2 mm. wide. Unfortunately, the superior and inferior mesenteric veins were severed close to their junction so that we do not know the pattern of their tributaries. The splenic vein left the spleen as a comparatively small vessel that soon became dilated, passing slightly downward and over the calcified aneurysms to empty into the portal vein. The main dilated portion of the splenic vein was continuous with a large tortuous anastomotic channel that extended beneath the left leaf of the diaphragm, passing downward

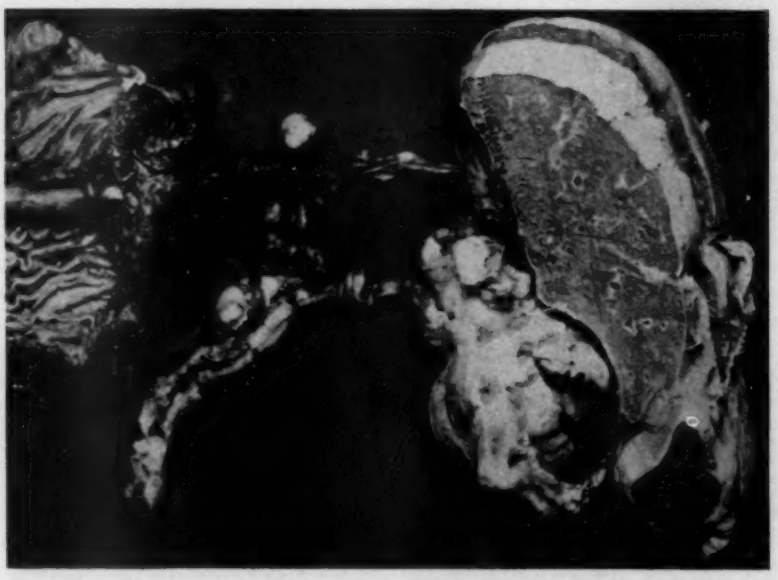

Fig. 2.—Gross specimen showing the spleen and the multiple aneurysms of the splenic artery.

until it ended in the left renal vein. As will be seen from the following measurements, stenosis of the portal vein with dilatation of its tributaries distal to the stenosis was present.

	Circumferences, Mm.
Portal vein above the pancreas	25
Portal vein in the pancreas, at the calcified ring	18
Combined superior and inferior mesenteric veins	30
Terminal ends of the splenic vein	28
Middle of the splenic vein	40
Anastomotic channel between the spleen and the left kidney	35 to 50

Between the splenic vein and the greater curvature of the stomach were five dilated veins, the vasae breviae. They connected with visible, easily palpated

varicose veins located in the wall of the stomach. The overlying gastric mucosa was eroded away, exposing the lumen of one varix. Some slightly dilated veins were present about the cardiac end of the stomach, and one large vein was traced the entire length of the esophagus. Both ovarian veins were dilated, and a plexus of dilated veins surrounded each ovary. The vena cava was patent throughout. The aorta showed surprisingly little atheromatous change for a woman of this age.

Red marrow was found in the middle third of the femur.

Histologic Examination.—The liver revealed an intact structure. The sinusoids were slightly dilated, with mild compression of the cords of hepatic cells. This was most marked about the central veins. Cloudy swelling of the parenchymal

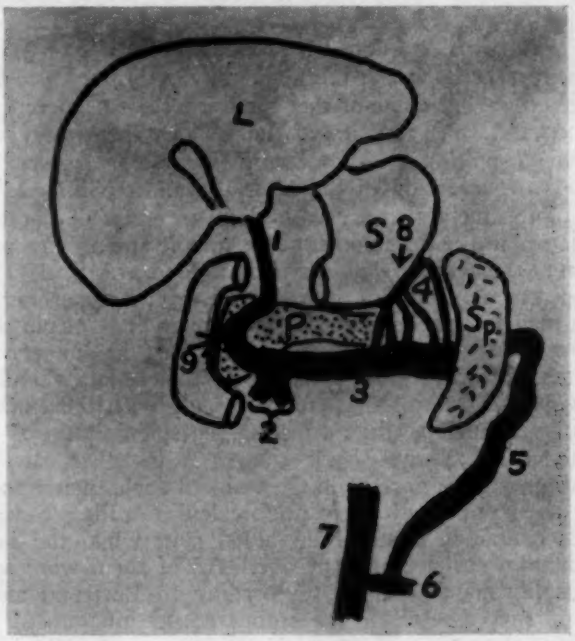

Fig. 3.—Outline of the portal venous system: *L*, liver; *S*, stomach; *Sp*, spleen; *1*, portal vein; *2*, mesenteric veins; *3*, splenic vein; *4*, vasa brevia; *5*, collateral venous channel; *6*, left renal vein; *7*, inferior vena cava; *8*, point of gastric bleeding; *9*, point of portal stenosis.

cells was present in the portal zones. A few of these cells contained small vacuoles, and a few others contained large vacuoles, presumably fat. There was not an increased amount of fibrous tissue about the portal structures. Here and there were small foci of polymorphonuclear leukocytes and degenerating liver cells.

The histologic structure of the spleen was rather indistinct. The lymphoid follicles were somewhat atrophic. Most of the sinusoids were widely dilated and empty. There was a general increase in the amount of stroma with a decrease in the number of pulp cells. Perifollicular hemorrhages were not noted in our slides. The blood vessels were dilated.

Final Diagnosis.—Stenosis and distortion of the intrapancreatic portion of the portal vein; dilatation of the splenic vein and formation of collateral channels; splenomegaly with perisplenic adhesions; ruptured varicose veins in the gastric wall; anemia, with hyperplastic marrow from the middle third of the femur; edema of the legs; bilateral hypostatic congestion of the lungs; bilateral hydrothorax and hydropericardium; decubital ulcers; multiple calcified aneurysms of the splenic artery.

COMMENT

Aneurysms of the Splenic Artery.—In 1924 Baumgartner and Thomas [2] collected 40 cases of aneurysm of the splenic artery in a review of the literature of the preceding fifty years. In 1929 Anderson and Gray [3] were able to collect 58 cases and reported a fifty-ninth. In 1939 Machemer and Fuge [4] collected 24 cases reported since 1929 and added a case. In 1940 Sperling [5] added 7 cases from the necropsy material of the department of pathology at the University of Minnesota. It appears, then, that less than 100 cases of this condition have been reported.

Machemer and Fuge [4] reviewed the various theories regarding the causes of this condition. No one etiologic factor seemed dominant. Atheromatous degeneration of the arterial wall, syphilis and embolism were some of the factors discussed. Remizov [6] stated that there were two main factors in the development of a splenic aneurysm: preliminary degeneration of the arterial wall and rise in blood pressure. The arterial degeneration was not present in our case. The hypertension in the splenic vein may have been transmitted through the splenic pulp to the splenic artery and may have played some part in the production of the aneurysms. However, Boyd [7] stated that there is a valvular mechanism in the splenic arterioles where they unite with the ellipsoids such that venous hypertension, while affecting the splenic pulp, is not transmitted to the splenic artery.

Calcification may be present in the walls of such aneurysms. In our case all the aneurysms had fairly thick calcified walls. Several of the smaller ones contained postmortem blood clot, while the larger ones contained antemortem clot of some age. All of them were surrounded by numerous fibrous adhesions. According to Bertrand and Clavel,[6] aneurysms of this type could be compared to inflammatory tumors, creating around themselves many thick adhesions to the posterior surface of the stomach, pancreas, colon and spleen. In the case being reported, these adhesions bound the aneurysms to each other and to the spleen, the other organs being free.

Sperling [5] in 1940 reported a case as the fourth case in which the correct preoperative diagnosis of this condition was made. In 3 of the 4 cases the condition was diagnosed by detecting the calcified walls of

2. Baumgartner, E. A., and Thomas, W. S.: Surg., Gynec. & Obst. **39**:462, 1924.

3. Anderson, W., and Gray, J.: Brit. J. Surg. **17**:267, 1929.

4. Machemer, W. L., and Fuge, W. W.: Arch. Surg. **39**:190, 1939.

5. Sperling, L.: Surgery **8**:633, 1940.

6. Cited by Machemer and Fuge.[4]

7. Boyd, W.: Textbook of Pathology, ed. 2, Philadelphia, Lea & Febiger, 1934, p. 796.

the aneurysms in the roentgenograms. In the fourth case, a correct diagnosis was made on the basis of pain in the left upper quadrant of the abdomen with a systolic bruit over a palpable tumor mass and a pulsating filling defect in the greater curvature of the stomach. Figure 1 shows the roentgenogram of the aneurysms and spleen in the case being reported. Detection of these shadows before death in this case would only have added to the clinical confusion.

The treatment of the condition consists in surgical removal of the aneurysms with splenectomy. Sperling [5] stated that the case he reported was the twelfth in which an aneurysm of the splenic artery had been successfully removed surgically. In our case the aneurysms were really incidental findings, since it was Banti's disease that caused all the patient's symptoms and finally her death.

Aneurysms of the splenic artery tend either to rupture or to erode into a neighboring organ. They are one of the less common conditions that must be considered in the differential diagnosis of intra-abdominal hemorrhage. As shown in this case, they may be without demonstrable symptoms.

Chronic Congestive Splenomegaly (Banti's Disease) Without Hepatic Cirrhosis Due to Stenosis of the Portal Vein.—At first we were unable to explain the finding of a noncirrhotic liver in what would otherwise be a case of late Banti's disease. However, after encountering the observations and conclusions expressed by Thompson [1] regarding the genesis of the condition, the true nature of this case became apparent to us.

The following statements from his report contain the essence of his conception of this disease:

> 1. Direct or indirect evidence of portal vein hypertension exists as the common denominator in all cases of so-called Banti's disease or splenic anemia.
>
> 2. This portal hypertension in the presence of normal peripheral venous pressure results in the splenomegaly, the collateral circulation, and the esophageal varices.
>
> 3. A simple mechanical reason for this hypertension can be found in every case that can be adequately studied.
>
> 4. Hepatic cirrhosis exists in 68 per cent of our series as the obstructive factor. When cirrhosis is not present at the time of splenectomy it will not appear subsequently.
>
> 5. No clinical or hematological differences can be found between patients with congestive splenomegaly due to intra- or extrahepatic obstructions, except in cases of advanced liver disease when the clinical features of hepatic insufficiency will appear and assume preponderance.
>
> 6. The splenic histopathology is the same in all cases of congestive splenomegaly —it is similar in type to the changes described by Banti and others—there are no visible differences in the various groups.

In the case being reported, only indirect evidence of hypertension of the portal vein was available. This evidence consisted of dilatation of the portal vein and its tributaries distal to the stenosis and formation of an extensive collateral circulation between the portal and caval venous systems. There was no evidence that this patient had other than normal peripheral venous pressure. Consequently, sufficient gradient in pressure was present to permit establishment of an extensive collateral circulation.

After discovering Thompson's [1] report, we dissected out the portal venous system to see if there was an obstruction present as he insisted there must be (statement 3). This was possible since we had fortunately removed the spleen, pancreas and a small portion of the liver en masse in order to preserve the calcified aneurysms of the splenic artery. To our surprise and gratification, there was definite stenosis with distortion of the intrapancreatic portion of the portal vein. The caliber of the vein between the stenosis and the liver was normal, while that of the vein and its tributaries distal to the stenosis was greatly increased.

From the point of view of histology the dominant feature of the spleen in this case was the prominence of the sinuses. Boyd [8] stated that the number and the size of the sinuses constitute the most striking feature of his own material. We failed to find any periarteriolar hemorrhages. Neither was the fibrosis advanced. While these two points are not mandatory for a histologic diagnosis, we mention them for completeness.

An inexplicable feature is that this woman appeared to be in fair health until the sudden onset of gastric bleeding. Evidently the tremendous venous dilatation and splenic congestion were practically asymptomatic.

We discovered that in 1903 Trevor [9] reported a case having this unusual combination of Banti's disease and aneurysms of the splenic artery. The patient had atrophic cirrhosis of the liver, splenomegaly, perisplenic adhesions, splenic fibrosis and extensive dilatation of the branches of the portal vein as well as multiple calcified aneurysms of the splenic artery. He considered the case to be one of Banti's disease with the incidental finding of aneurysms, as in our case.

In 1924 Baumgartner and Thomas [2] reported a case of aneurysm of the splenic artery which in life was thought to be possibly a case of Banti's disease. The final diagnosis after necropsy was as follows: aneurysm of the splenic artery, compression of the splenic vessels with chronic passive congestion of the spleen, varices of the vasa brevia of the stomach, hemorrhage into the stomach and intestines and bronchopneumonia. The liver was not described, and from the final diagnosis one is led to understand that the obstructive factor here was compression of the splenic veins by the aneurysm.

SUMMARY

A case of mechanical congestive splenomegaly (Banti's disease) without cirrhosis of the liver and apparently caused by stenosis of the portal vein is reported. Death was due to recurrent hematemesis from ruptured gastric varices. Multiple calcified aneurysms (unruptured) of the splenic artery were incidental findings.

1103 Grand Avenue.
Trinity Lutheran Hospital.

8. Boyd, W.: Pathology of Internal Disease, ed. 3, Philadelphia, Lea & Febiger, 1940, p. 640.

9. Trevor, R. S.: Tr. Path. Soc. London **54**:302, 1903.

TRAUMATIC HEMORRHAGE INTO THE PITUITARY GLAND

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Hemorrhage into the substance of the pituitary gland following trauma has not been described frequently. Simmonds [1] stated ". . . in my series of cases, which include a number of cases of fracture of the base of the skull, I have observed hemorrhage into the capsule of the hypophysis but not into the substance of the gland itself." Schmorl [2] observed hemorrhagic necrosis of the hypophysis in a case of fracture of the base of the skull. In 1912 Cushing [3] reported 3 fatal cases of cranial injury in which extravasations were found in the substance of the posterior lobe. Although congestion is not uncommonly seen in the anterior lobe, the pars posterior is much less frequently involved.

REPORT OF A CASE

A 35 year old white man was thrown from a moving car and landed on his head. He was rendered unconscious and remained unconscious until the time of his death three hours later. On his admission to the hospital the pulse rate was 56; the beat was forceful but irregular. The blood pressure was 188 systolic and 80 diastolic. Respirations were of Cheyne-Stokes character, deep but with normal rate. The respiratory passages were partially obstructed by aspirated vomitus. Percussion of the skull produced a cracked pot note, particularly in the right temporoparietal region. There was a hematoma under the scalp over the right supraorbital ridge. There was ocular proptosis and beginning ecchymosis about the right eye. The pupils were found to be normal in size, equal and regular, but did not respond to light. No extraocular movements were seen; the gaze was directed forward. Profuse bleeding from the nose was noted, and the discharge was thin, suggesting dilution with cerebrospinal fluid. No blood appeared in the aural canals. All extremities were markedly atonic, and both the deep and the superficial reflexes were almost absent when first tested. Pathologic toe and finger signs were absent. No evidence of acute trauma was to be found on examination of the chest, abdomen or extremities.

The patient was promptly given 50 cc. of 50 per cent dextrose intravenously, which had little effect on the vital signs. Because of continuing unfavorable progress, a lumbar puncture was done, and 30 cc. of grossly bloody cerebrospinal fluid under markedly increased pressure was removed. Shortly after the lumbar

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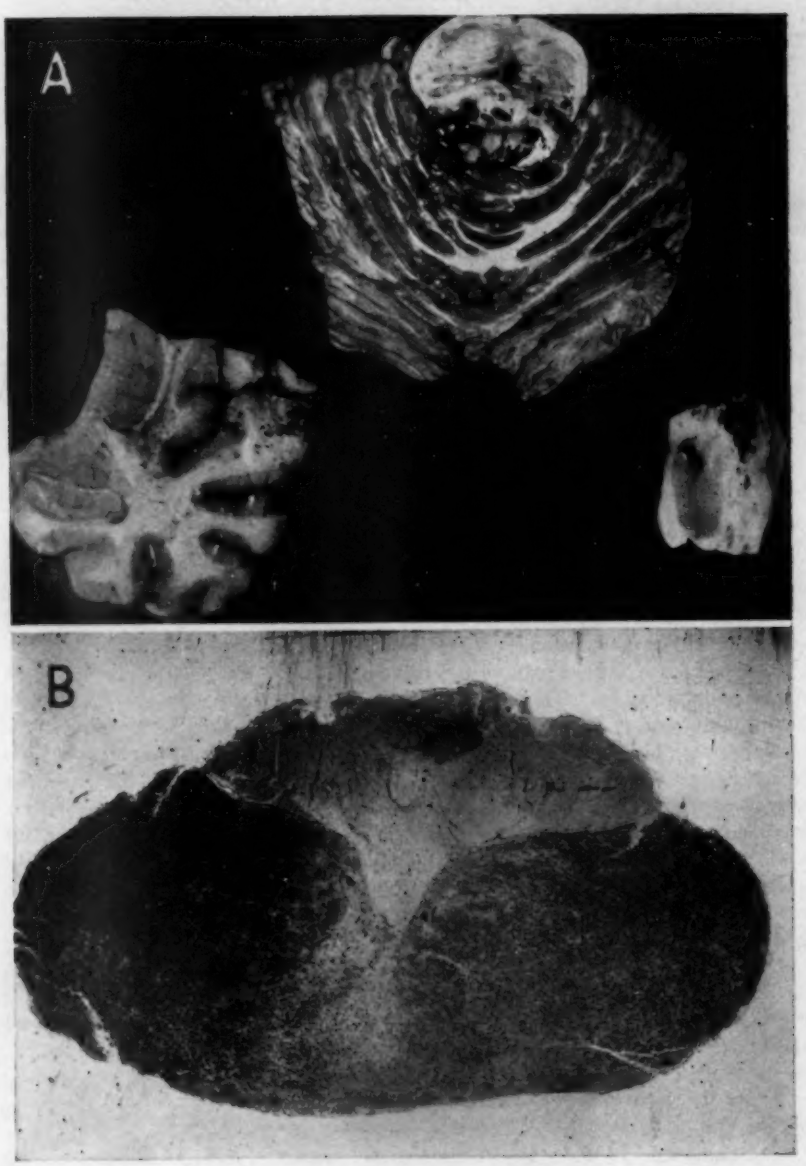
1. Simmonds, J. P.: The Pathological Anatomy and Histology of the Hypophysis, in Barker, L. F.: Endocrinology and Metabolism, New York, D. Appleton and Company, 1922, vol. 1, p. 770.

2. Schmorl, G.: Die pathologischen-histologischen Untersuchungsmethoden, ed. 4, Leipzig, F. C. W. Vogel, 1907; ed. 15, 1928.

3. Cushing, H.: The Pituitary Body and Its Disorders, Philadelphia, J. B. Lippincott Company, 1912, p. 173.



A, hemorrhages in the cerebrum and the pons.
B, hemorrhage in the posterior lobe of the pituitary gland.

puncture, the patient's blood pressure fell to 120 systolic and 80 diastolic; the pulse became more regular but continued to increase in rate, while the respirations became more irregular and labored. The patient's condition rapidly became worse until respirations ceased—two and one-quarter hours after the accident. The heart, however, continued to beat, and the blood pressure was recorded at 80 systolic and 0 diastolic. Administration of a 95 per cent oxygen–5 per cent carbon dioxide mixture with a respirator was continued, together with administration of coramine (a 25 per cent solution of pyridine betacarboxylic acid diethylamide) and dilatation of the rectum, but the heart action ceased forty-five minutes later.

Autopsy, at the coroner's morgue, was confined to the head. No extradural clot was seen over the convexity. However, areas of subdural hemorrhage were found over both temporal lobes, the film of blood on both sides measuring from 2 to 3 mm. in thickness. There were slight lacerations of both frontal lobes and of the inferior portion of the left temporal lobe.

Serial coronal sections, taken through the brain, immediately showed scattered small hemorrhages in the gray and the white matter of the cerebrum (*A* in figure) and more extensive extravasations in the region of the lacerations mentioned. The cerebral ventricles were free of blood. Sections through the pons showed many areas of hemorrhage, which measured up to 6 mm. in diameter, particularly in the tegmental region. Sections of the medulla and of the upper cervical part of the cord were free of hemorrhages. The cerebellum showed no gross evidence of injury.

There were numerous linear fractures in the base of the skull: several extended from the right parietal region downward into the middle fossa and thence medially to the sella turcica; others passed through the cribriform plate.

PATHOLOGIC CHANGES IN THE PITUITARY

Gross Examination.—The capsule was somewhat discolored by a thin layer of extradural blood clot. The gland weighed 537 mg. and measured 14 by 10 by 5 mm. The posterior lobe measured 8 by 6 by 4 mm. The capsule was stripped before weighing the gland. Serial sections were cut in the horizontal plane. Gross areas of hemorrhage measuring up to 3 mm. in diameter were found throughout three fourths of the sections cut serially through the posterior lobe of the gland.

Microscopic Examination.—The serial sections showed several small areas of hemorrhage beneath the capsule in the parenchyma of the posterior lobe. The largest hemorrhage seen grossly was deep in the substance of the posterior lobe itself (*B* in figure). In addition to this large hemorrhage, there were also numerous small areas of hemorrhage scattered throughout the parenchyma of the posterior lobe. No hemorrhage was seen in the anterior lobe of the gland. The loose tissue of the capsule was infiltrated with red blood cells. The cells of the posterior lobe showed no recognizable changes. Many cells containing brown granular pigment were seen in the sections stained with hematoxylin and eosin. The source of the hemorrhage could not be traced to the large or medium-sized blood vessels in the posterior lobe. It had apparently resulted from capillary extravasation in the contusion of the substance of the pituitary gland associated with the basal skull fracture which had penetrated the sella turcica. In the mid-portion of the posterior lobe there was a cystic structure which contained a colloid-like material. This cyst had no epithelial lining and measured 0.5 mm. in diameter. It appeared to be a remnant of the hypophysial fissure, i. e., a derivative of the glandlike structure so frequently seen in the region of the pars intermedia.

In 1904 Madelung [4] and later Maronon and Pintos [5] reported cases of pituitary insufficiency secondary to the lodgment of a rifle bullet in the sella. Reverchon, Worms and Rouquier [6] demonstrated sclerosis and atrophy of the hypophysis some months after fracture into the sella turcica with hemorrhage. Hypopituitarism secondary to similar severe injury of the head has also been described by Bleitreu,[7] Cushing,[8] Benedek and Angyal.[8] It seems to be agreed that because of the rich blood supply and vulnerable location, the pituitary gland is injured more often than is believed, and in many cases it is the resorption of intraparenchymatous hemorrhages which gives rise to transient hypophysial deficiency.

SUMMARY

We report a case of basal skull fracture extending to the sella turcica in which postmortem examination revealed, among other findings, hemorrhage into the pars posterior of the pituitary gland. We record this case not only because of its scientific value but because it shows the importance of a complete examination in cases of severe cranial trauma. Posttraumatic changes in the pituitary gland should always be looked for clinically and pathologically in cases of fracture of the base of the skull, as injury of this gland definitely increases the gravity of the prognosis.

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Indiana University Medical Center, Indianapolis.
Henry Ford Hospital, Detroit.

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5. Maronon, G., and Pintos, G.: Nouv. iconogr. de la Salpêtrière **28**:185, 1916.

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METASTATIC CALCIFICATION IN OSTEITIS DEFORMANS (PAGET'S DISEASE OF BONE)

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Metastatic calcification does not seem to have been previously reported as occurring in osteitis deformans (Paget's disease of bones), and this is not surprising in view of the fact that calcium metabolism is not abnormal in osteitis deformans. Thus Gutman, Tyson and Gutman [1] made analyses of the blood in 76 cases of Paget's disease and reported values for calcium within the normal range of 9 to 11 mg. per hundred cubic centimeters in 65, and values for inorganic phosphorus in the normal range of 2.7 to 4.5 mg. per hundred cubic centimeters, except in 6 cases with high nonprotein nitrogen values. Sugarbaker,[2] on reviewing 51 cases of osteitis deformans, reported that "a negative calcium and phosphorus balance is generally present in the earliest and more active stages of the disease and a positive balance in the later and less active phases, but the blood calcium and phosphorus levels are normal throughout." "In our series the blood calcium range was 9.0 to 10.6 mg. per cent, the blood phosphorus range was 1.72 to 4.54 mg. per cent, with averages of 9.7 and 3.5 mg. per cent respectively." "Any constant variations in the serum calcium depending on the stage of the disease we were unable to discover." "Calculi are not common, occurring in four of the present series," of which two were biliary and two renal.

The presence of hypercalcemia, which might lead to metastatic calcification, is rare, and usually due to some complicating condition. Thus in the series of Gutman, Tyson and Gutman was 1 case in which the calcium exceeded 11.5 mg. per hundred cubic centimeters, a finding which was associated with bronchogenic carcinoma; here there were some calcified bodies in the renal tubules, but no metastatic calcification was recognized. Albright, Aub and Bauer [3] reported a case in which osteitis deformans was associated with hyperparathyroidism in a woman who had a serum calcium level of 13.2 mg. and a phosphorus level of 2.2 mg. per hundred cubic centimeters, but the association is regarded as a pure coincidence. The same assumption of coincidence is made by Gutman and Parsons [4] in reporting 3 cases of hyperparathyroidism associated with Paget's disease. Repeated high calcium values rule out osteitis deformans, according to Gutman and Kasabach.[5] Schmorl [6] reported necropsies in 138 cases of Paget's disease with no alteration in the parathyroids, and made no mention of metastatic calcification in

From the Department of Pathology of the University of Chicago and the Otho S. A. Sprague Memorial Institute.

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any of them. It therefore seems desirable to place on record a case of osteitis deformans in which extreme metastatic calcification was observed.

REPORT OF A CASE

The patient, a boiler maker, 59 years of age, entered an outlying hospital on April 27, 1933, complaining of enlargement of the head for six to seven years, pain and stiffness in the left hip and knee for three years, crook in an arm for six to seven years, pain and stiffness in the left hip and knee for three years, and difficulty in reading for eight months. He first noted an enlargement of the head at 50 years of age, when he had to buy a hat of larger size than he was accustomed to. Two years later he began to have slight pain in the left leg and arm. Physicians diagnosed rheumatism, but the condition did not respond to the treatment instituted, so the patient had treated himself with liniments, baths and pads. He secured a job requiring considerable climbing but had to give this up because of pain in the left knee joint. During the three years preceding examination his head had continued to increase in size so that his size of hat, formerly a 7, had gradually increased to size 8. For four or five years he had noted gradual bowing of the legs, accompanied by pain in the left leg. For two years the pain had been progressing, particularly in the hip. For three or four years there had been weakness because of which the patient could walk only three or four blocks at a time. He weighed about 140 pounds (63.5 Kg.) on entrance; in 1923 he weighed 160 pounds (72.6 Kg.). Eight months prior to entrance he began to have difficulty in reading and occasionally noted blurring of vision. He then went to the Illinois Eye and Ear Infirmary and was referred to the hospital.

Inquiry by systems revealed slight dyspnea and palpitation on exertion and definite weakness, slight edema of the left leg, backache at times, nocturia (micturition once a night), headache and slight dizziness on bending over, observed for three or four months, and poor hearing. The family history was noncontributory.

The patient was poorly nourished and poorly developed. He was not acutely ill but showed deforming features as follows: dilated and tortuous veins in the scalp, which was stretched and shining; absence of hair; very large size of the head, with the brow wide and overhanging, the eyebrows arched, a deep pocket beneath each eye, a protruding jaw and teeth in the lower set protruding beyond those in the upper. The head presented the appearance of an inverted triangle with the apex at the chin. The pupils were equal and regular, and reacted to light and accommodation. There was old chorioretinitis with marked defects in the retina. The chest was barrel shaped with flared lower ribs and an enlarged sternum, which on percussion gave a sound suggesting that it was not solid. There were rales in the chest both anteriorly and posteriorly. There was kyphosis of the back. The extremities showed bowing of both radiuses and ulnas, also of both tibias and a marked anterior and lateral bow in the right leg.

There was marked diminution of visual acuity. Fingers could be counted at 10 feet (305 cm.) with the right eye and at 2 feet (61 cm.) with the left eye. There was bilateral absence of the achilles reflex. The heart showed a systolic murmur at the apex, transmitted to the axilla, and some enlargement. The blood pressure was 150 systolic and 80 diastolic.

The blood plasma chemistry determinations were as follows:

Date	CO_2	p_H	Ca	P	K
4-28-33	55 cc.	7.45	11.83 mg.	3.74 mg.	29.12 (?) mg.
4-29-33	59	7.3	10.15	4.55	17.88
5- 1-33	56	7.4	10.10	4.17	16.69

The urine was normal. No blood count was recorded. While the patient was in the hospital, his temperature was normal or slightly below normal, 97.2 to 98.8 F. The pulse rate ranged between 70 and 110, usually between 90 and 100, and the respiratory rate was 20 practically all of the time. The patient was up and about the hospital every day, slept well and ate a general diet.

Viosterol "ergosterol activated 10,000 times" was given in 10 minim (0.62 cc.) quantities once daily, in the morning, beginning on May 12 and continuing until the day of discharge, May 26, 1933. No determinations of blood calcium were made after the viosterol was given. The roentgenograms showed lesions characteristic of Paget's disease: marked thickening of the skull bones, a heavy mandible with markedly varying density, giving a "moth-eaten" appearance to the bones of the calvarium especially. Bones in the extremities had a similar appearance, though less marked, some showing also a slight waviness of the bone margins. There was marked bowing of the tibia, with varying density as in other bones. There were marked widening and some variation in density of the lumbar vertebrae, particularly, and scoliosis involved the lower thoracic and most of the lumbar vertebrae. The pelvic bones and the heads and necks of the femurs showed extreme variation in density, with the same moth-eaten appearance as the skull. There was marked enlargement of the bones of the pelvis, the bones of the extremities, and the skull, also of the lumbar vertebrae. Nothing was known of the patient's subsequent history until about July 25, at which time a physician was called, who thought bronchopneumonia was developing, and believed this to be the cause of death on July 29. The opportunity to perform the necropsy in this case was given us by Dr. C. G. Sachtleben.

Necropsy.—External Appearance: On palpation the skull was irregularly flattened in places, chiefly over the parietal regions, and was depressed somewhat over the frontoparietal junction on each side, the depression being more marked on the left side. There seemed to be small nodular irregularities in the skull on the left side and, on external palpation, over the anterior parietal and the posterior portions. The body was 59 inches (150 cm.) long and weighed 112 pounds (50.5 Kg.). The circumference of the head was 62.5 cm. The head measured 20 cm. in its greatest width, which was the interparietal dimension, and 20.5 cm. in the anteroposterior dimension, from the level of the supraorbital ridges to the occipitoparietal junction. The anterior half of the scalp was almost devoid of hair. There was a definite anterior projection of the mandible, which appeared somewhat large relative to the face. The maxillary bones were somewhat prominent. There was some asymmetry of the face, the left half appearing slightly lower than the right; this was due to the pointing of the mandible slightly to the right and downward as compared with the right side. In the anterior and posterior margins of each axilla, over the right lateral thoracic region and over the left posterior thoracic and upper lumbar region the skin was thickened and appeared slightly nodular but, on pinching, seemed to contain a soft material which could be altered in shape on pressure. The two largest areas measured approximately 17 cm. in diameter. The superficial lymph nodes were not palpable. The anterior superior spines of the ilium, as well as the crest, seemed extremely thickened on palpation. There was moderate middorsal kyphosis. There was a slight flaring of the medial portions of the lower ribs and costal cartilages, more prominent on the right side. There was marked bowing of the bones of the forearms, the convexity being lateral. This was a little more marked on the right than on the left. The bones of the hands seemed symmetric. There was marked lateral and also anterior bowing of the lower extremities, slightly more marked in the tibias than in the femurs and in the left

slightly more than in the right. There was definite thickening of the tibias on external palpation, that in the left in the midportion feeling rather rough and enlarged fusiformly in its middle third. The sternum was thickened and measured approximately 3 cm. in thickness.

Viscera: The pericardial sac contained about 30 cc. of clear yellow fluid. There was rather marked dilatation of the right atrium. The right ventricle was in diastole; the left ventricle and atrium were in systole.

The epicardium contained a moderate amount of adipose tissue. It was diffusely thickened and slightly opaque, more so along the course of some of the vessels, which were slightly tortuous but not sclerotic. There was slight focal thickening of the endocardium of the right atrium. There was some fibrous thickening along the attachments of the chordae tendineae to the tricuspid leaflets and the tips of the papillary muscles. There were a few small focal thickenings of the endocardium just beneath the pulmonary orifice. The pulmonary cusps showed no change. The endothelium of the left atrium was somewhat thickened, and in the medial and posterior wall there were numerous very thin subendothelial calcified plaques, some of them almost 1 cm. in diameter. The mitral orifice admitted two fingers. The anterior leaflet projected into the orifice and appeared to occlude it partially, being semirigid because of calcification. The mitral orifice measured 9 cm. in circumference. The posterior leaflet of the mitral valve exhibited a few focal areas of calcification at its base. The posterior portion of the annulus fibrosus was calcified. There was a calcific bar, 15 mm. in length and 2 mm. in diameter, across the tips of four papillary muscles to the posterior leaflet of the aortic valve. In the substance of, and in the inferior surface of, the anterior mitral leaflet, 5 mm. from the free margin, which itself showed no change, there were several protrusions of calcified material, as mentioned, one bar of which was 15 mm. in length and 4 mm. in width and extended into the base of the left anterior aortic cusp. In the myocardium of the interventricular septum there was an irregular area, measuring 2 cm. by 8 mm., which was almost white. There were numerous similar but smaller areas scattered through the myocardium. They were present in the apex also but to a less degree. They did not feel softer or firmer than the more normal muscle about them. The heart weighed 360 Gm. In the right anterior and in the posterior aortic cusp there was marked calcification in the base and midportion. There was also a protruding bar of calcific material, 2 mm. in diameter, extending from the right commissure to the midportion of this leaflet. The sinuses of Valsalva showed no change.

The left coronary artery showed only slight sclerosis at its beginning and along its branches, with almost no calcification. It was somewhat tortuous, as were its branches. The right coronary artery resembled the left. The ascending aorta was of normal elasticity and appearance. There were several calcified plaques measuring up to 1 cm. in the arch and several similar areas of sclerosis and of atheromatous change in the descending portion. The inferior vena cava and the portal vein showed no changes. The abdominal aorta showed moderate sclerosis with a few calcified plaques. The splenic artery and vein and the renal arteries and veins showed no change.

The lungs were expanded and filled the pleural cavities, which were dry and free of adhesions. Both lobes were firm throughout but on pressure were slightly crepitant. The lungs stood up as rigid organs, and on section the tissue was white and appeared very granular, almost simulating soft bone with very fine pores. The tissue was not friable. There was apparently a diffuse fibrosis of the alveolar walls, because air spaces appeared as fine pores. A small quantity of blood-tinged

fluid could be expressed from the posterior portion of the lower lobes, and all bronchi contained a varying amount of mucoid and slightly purulent material. The mucosa, however, did not show any change except perhaps very slight hyperemia. In the apex of the left lung and beneath the puckered pleura there was a cavity 1.5 cm. in diameter which had a thin wall of anthracotic fibrous tissue. There were numerous other smaller subpleural cavities, the pleura being rather thin and distended by air. There were similar cavities in the apex of the right lung. There was no calcification in any of the mediastinal lymph nodes. The right lung resembled the left in all details. The right lung weighed 1,070 Gm. and the left 1,400 Gm. The density of both lungs was increased posteriorly.

The other viscera, including the brain, showed no changes of significance. There was no visible calcification in the kidneys, and no calculi. The thyroid, hypophysis and parathyroids appeared to be of normal size and structure.

Skeleton: When the scalp was reflected over the depressions described in the frontal and parietal region, the bone in the depressions described was finely nodular and could be pierced easily with a scalpel. The anterior portion of the calvarium in cross section measured 19 to 24 mm. in thickness and laterally 17 mm. on the left side and 5 mm. on the right, while posteriorly it measured 15 mm. The inner table appeared softer than usual and slightly more rough than normal. The cancellous portions of the thicker places were more porous and softer than usual. The bone in the floor of the anterior fossae was reddened diffusely and had proliferated so markedly that the floor of the anterior fossa was elevated slightly into the cranial vault. There was definite widening of the corpus of the sphenoid, separating the optic nerves by a distance of 2 cm. The orifices for the optic nerve were transverse slits rather than round, and the nerves appeared of normal width but flattened. There was marked widening of the sphenoid in the base of the sella turcica, the pituitary fossa being of normal dimensions. There was not much distortion of the posterior fossa, though there was marked thickening of the occipital bone. The sternum was thickened and measured approximately 3 cm. in thickness. The heads of most of the ribs appeared enlarged. There were exostoses and some lipping of the vertebrae. There was slight middorsal kyphosis and slight left scoliosis. Section of the vertebral bodies disclosed increased porosity of their substance. The right iliac crest was 2 to 2.5 cm. in width, as was the left. The left femur at the upper end showed coxa vara, the angle formed by the neck and the shaft being very close to a right angle. The articular cartilage was thinned and discolored bluish peripherally and was also markedly thinned about the attachment of the ligamentum teres, with slight marginal lipping. The shaft of the femur was distinctly thickened, being one and a half times the diameter of the normal bone. This was particularly marked in the lower third of the shaft. The cortical surface of the bone was irregularly roughened throughout. The articular cartilage over the lower end of the femur showed marked erosion at the weight-bearing portion and lipping. There was increased anteroposterior bowing of the shaft. The weight of the bone was 1,640 Gm. in the fresh state. The left tibia weighed 1,000 Gm. and showed the same changes of the articular cartilage. The shaft was generally enlarged, the diameter of the middle third being 5 to 6 cm. There was marked increase in the anteroposterior bowing, with the apex at the middle third. The normal triangular shape of the shaft had been lost, and the bone had assumed a more rounded shape. The tibial crest, particularly the upper two thirds, had been rounded. The surface of the bone was irregularly roughened by plaquelike bone formations. The left fibula weighed 150 Gm. and showed only slight enlargement, generally with moderate roughening of the surfaces of the bone.

Microscopic Examination.—In addition to the bony changes of Paget's disease, the tissues presented a most striking picture of extensive metastatic calcification. Especially the lungs, which grossly had appeared as rigid, solid organs and which when cut resembled bone, presented a striking picture. In addition to extensive bronchopneumonia, which caused death, they showed marked calcification of the alveolar walls as well as fibrous thickening. In large areas almost no alveolar walls had escaped infiltration with calcium. The pulmonary arteries had escaped, but the small veins were heavily calcified, often appearing as a ring of calcification. This was evidence of the supersaturation of the blood with calcium produced when the carbon dioxide was lost through respiration and the calcium was precipitated in the carbon dioxide–poor, venous, pulmonary circulation.

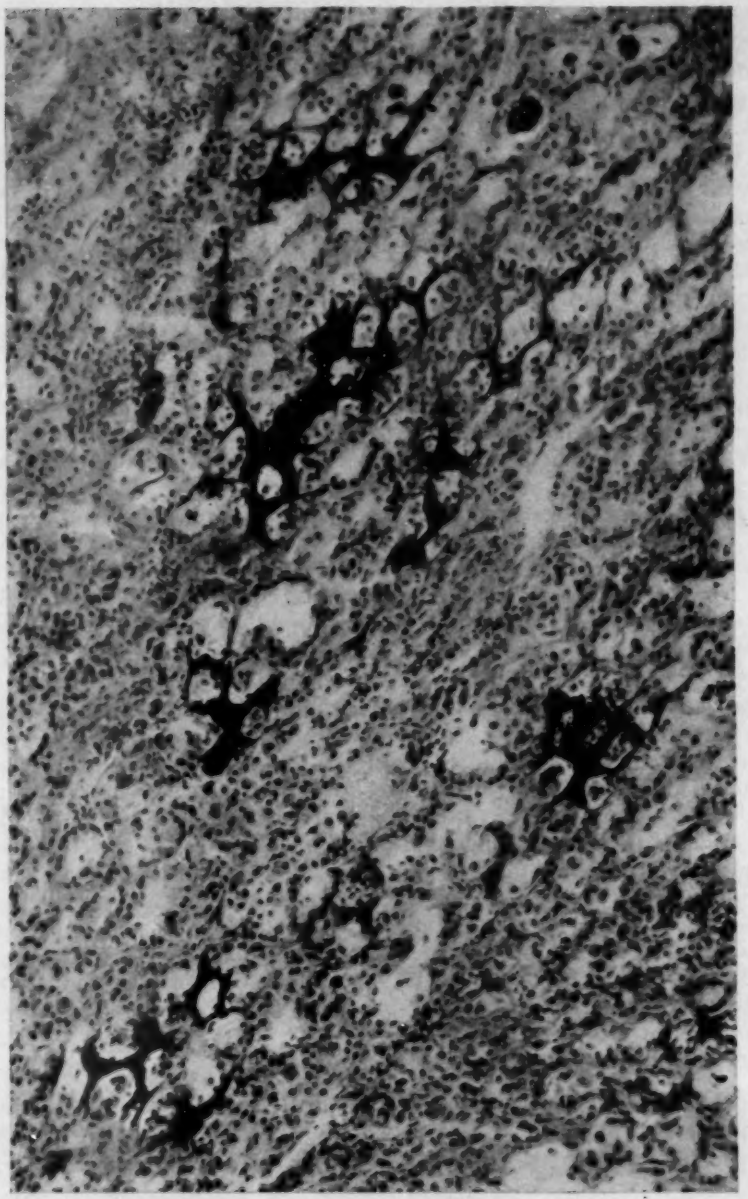
Of similar significance was the precipitation of calcium found in the left side of the heart, for we found the most extensive calcification in the left atrium. The calcification of the aortic cusp was apparently not related to the metastatic calcification, for it showed ossification and marrow formation, and the calcification of the aorta was presumably merely senile change. But the condition was quite different in the left atrium, where a thick layer of calcium had been laid down in the intima from the supersaturated blood. Also the condition in the stomach was characteristic (fig.), for often about the acid-secreting glands there was found a dense ring of calcium, deposited where the excretion of hydrochloric acid had left a compensatory degree of local alkalinity, while the glands of the pylorus had no such calcium deposit. In the kidney also, where secretion of acid urine had produced local alkalinity, there was a heavy deposit in the tubules and, to a less degree, in the arteries.

Less easily explained was the deposition of calcium in the skin, which showed a heavy focal, calcium deposit in the subcutaneous tissues surrounded by a marked fibroplastic reaction, in which many young fibroblasts and foreign body giant cells were numerous.

The parathyroids were entirely normal.

The bones showed the typical changes of Paget's disease. In places the spicules were very thick, relatively close together and well decalcified. In others the spicules were very irregular in size, had ragged margins and were poorly decalcified. The spaces were filled with a loose structure of branching fibroblasts, and in some places there were small groups of myeloid cells. There were numerous multinucleated giant cells, some of which were producing lacunar absorption of the bone. The marrow was chiefly composed of loose fibrous tissue. The trabeculae were of extremely irregular structure, generally characterized by wavy lines of denser calcification. There were many evidences of absorption of normal bone and deposition of abnormal calcified material. There were also areas where the bone structure had been almost completely absorbed and replaced either with fibrous marrow or atypical calcific deposits.

The anatomic diagnosis was: advanced osteitis deformans (Paget's disease) involving the skull, the bones of the forearms and of the lower extremities, the vertebral column, the heads of the ribs and the pelvic bones and the sternum, with slight middorsal kyphosis and left scoliosis; extensive metastatic calcification of the lungs, of the endocardium of the left atrium, of the kidneys, of the gastric mucosa and of focal areas of the skin; extensive calcification of the anterior leaflet of the mitral valve and of the cusps of the aortic valve; bilateral organizing bronchopneumonia; focal parenchymatous degeneration of the myocardium; cerebral arteriosclerosis; mucopurulent bronchitis; edema of the lungs



Gastric mucosa involved in metastatic calcification, showing heavy calcium deposits about acid-secreting glands; × 155.

and of the tracheobronchial lymph nodes; slight pitting edema of the ankles; slight cloudy swelling of the liver and kidneys; healed bilateral apical tuberculosis (pulmonary) with multiple small cavities; focal fibrous peritonitis and focal scarring of the capsule of the liver; thrombosis of the inferior hemorrhoidal veins.

COMMENT

Why did such extensive metastatic calcification develop in this man in view of the fact that Paget's disease of bones has not hitherto been associated with such a condition? One explanation might be the administration of viosterol. This is especially probable in view of the metabolic studies in Paget's disease by London and Bernheim.[7] They administered calcium gluconate intravenously in 17 cases and found the resulting curves to be flatter than normal, indicating an increased affinity of bones "and other tissues" for calcium or a decreased affinity for calcium on the part of the blood. "The former interpretation is in line with the metabolic studies reported by several investigators, showing a retention of calcium by the body in Paget's disease of bone," and the statement of Moehlig and Adler[8] that the calcification of goiter and fibroids, as well as renal calculi, associated frequently with arteriosclerosis, would indicate that the bone lesions surrender calcium to the soft tissues. They also stated that "the feeding of calcium, viosterol, and vitamin D in this disease would seem to be contraindicated as this has a tendency to increase deposition of calcium in the soft tissues. We have not been able to improve the bone condition or arrest its progress by these products."

Unfortunately, no blood calcium analyses were made after the administration of viosterol was begun, for possibly this administration was responsible for the metastatic calcification. The patient was given approximately 5,000,000 U. S. P. units in the course of fifteen days, and from what is known of Paget's disease it would appear that the calcium is more easily mobilized from the bones than in normal persons. When this is taken together with the tendency toward calcification of the soft tissues it would seem rational to expect that massive doses of vitamin D would lead to more diffuse calcification in patients with Paget's disease than in normal persons. No other explanation for the metastatic calcification presents itself. We can find no other case of Paget's disease with metastatic calcification in the scanty literature on metastatic calcification or in the more abundant literature of Paget's disease.

SUMMARY

A case of Paget's disease associated with most extensive metastatic calcification is reported. This combination seems to be unique as far as a search through the literature reveals. A possible explanation lies in the fact that viosterol was administered.

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General Reviews

EFFECTS OF RADIATION ON NORMAL TISSUES

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TABLE OF CONTENTS

I. INTRODUCTION

In almost every therapeutic application of radiation, normal tissues are affected as well as the intentionally irradiated focus of disease; indeed, no small part of the response of a neoplasm to radiation is secondary to changes in the adjacent normal tissues.

This review will be limited to the effects on normal animal and human tissues of therapeutic radiation from the more important sources —roentgen rays (produced at low as well as high voltages), radium, radon, neutrons and certain of the temporarily radioactive isotopes. The effects on neoplasms and inflammatory processes will be omitted. Material from several accessible reviews,[1] which contain data on radiation-induced changes in normal tissues, and from some of the articles covered therein, will not be included except as needed for continuity

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1. Colwell and Russ. Desjardins. Duggar. Rolleston. Warren.

of discussion. References to the literature in the text and in the footnotes may be found in complete form in the bibliography at the end of the chapter in which they occur. When it is necessary to distinguish between articles by the same author, dates will be given in parentheses, accompanied by a letter of the alphabet if the author has published two or more articles in the same year.

Roentgen rays are short electromagnetic waves, as are the gamma rays from radium and radon. Alpha particles (helium nuclei with two positive charges) and beta rays (electrons) are usually screened out during radium and radon therapy, and hence are of little practical importance. The alpha particles have been stated to be about a hundred times more effective biologically than the beta rays (Lazarus-Barlow and Beckton). Many temporarily radioactive isotopes give both beta and gamma radiation, and some, such as phosphorus, only beta radiation. The biologic effects of these forms of radiation are qualitatively similar.

As yet, no good standard exists for comparing the activity of artificially radioactive isotopes with that of other types of therapeutic radiation. Although their radioactivity may be measured, as by electroscopes or Geiger counters, and is expressed as millicurie equivalents, this does not imply equivalence in biologic effect.

The first mention of the general biologic effect of artificially radioactive isotopes on cells was made by Lawrence and collaborators, who described diminution in the number of leukemic white blood cells as a result of the administration of radioactive phosphorus. The first reference suggesting the similarity in effect between radiation from radioactive isotopes, and that from other sources on normal cells was made by Warren and Gates in their study on radiation pneumonitis.

The effect of neutrons has not as yet been extensively studied, but it is suggested that in equivalent doses as determined with an ionization chamber they may have greater biologic effect than roentgen rays (Giles; Lawrence and Lawrence).

The effect of radiation on a given tissue is proportionate to the amount absorbed, not to the amount delivered. The radiant energy (photon) may traverse without absorption; it may, if it collides with an electron, be entirely expended (photoelectric effect); or it may, by removing an electron from an atom, expend only part of its energy and continue in a changed direction, in which case the wavelength will become longer (Compton effect).

A large part of the effect of radiation on tissue is believed to be due to the rays that are scattered within the tissue volume. These moderate speed electrons are removed from the atoms by the electromagnetic waves. Since the number of electrons separated from the atoms for a given dose of radiation increases with the mass that is radiated, the size of the field of exposure is a most important factor as a result of the secondary radiation thus produced.

The rate of radiation is also significant. With a very low rate, regeneration will compensate for any biologic effect on somatic cells and hence no result will be apparent. One-tenth roentgen (r) per day is accepted as a safe tolerance dose for workers in contact with radiation. Heavy dosage given at a rapid rate may destroy all tissues. At a lesser rate, however, the same dose will show selective action, damaging some tissues more than others. On this fact much of the radiation therapy of tumors depends.

As has been pointed out by Regaud and many others, variations in wavelength within the therapeutic range do not produce qualitative differences in cellular response. The character of this response is determined by the amount of radiation absorbed, the intensity of radiation and the area exposed. Much confusion as to the effects obtained with radiation is due to the failure of many investigators to specify the physical factors utilized. Within the range from 2 angstroms to 0.05 angstrom, variation in wavelength is apparently not a factor of importance other than in determining the degree of absorption and thus the depth of penetration. When small biologic test objects are used, the destructive effect within this range is commensurate with the number of roentgens delivered (Wood). When larger masses of tissue are involved, gamma radiation and supervoltage roentgen radiation produce less change in the skin than do rays of longer wavelength in comparable doses as determined with an ionization chamber (Failla, 1938). The variation in the number of roentgens at various voltages required to produce cutaneous erythema in man has been studied by Hudson and others. Only the intensity, however, and not the essential character of the cutaneous reaction, varies.

It is too often assumed that radiation universally affects the tissue which is exposed to it, and some investigators even have the impression that every particle within a field of radiation is subject to the radiation effect. This is far from the truth. For example, Crowther has calculated the chances of irradiation of molecules of air as follows: "Suppose that it were possible to pass, through the air of an ionization chamber, X-radiation at an intensity of 1 r per second continuously, day and night, for 500 years, we should still leave about one third of the molecules *unirradiated.*"

One pathologic effect of radiation was encountered before its nature was realized, and even before the discovery of either the roentgen ray or radium. For years the workers in the Schneeberg mines, in Germany, had been known to have a strangely prevalent fatal disease of the lung. This was later recognized as cancer of the lung, presumably caused by inhalation of radioactive particles from the ore of these mines (Pirchan and Šikl).

Within a year after the discovery of the roentgen ray, conjunctivitis, alopecia (Daniel) and radiodermatitis [2] were noted in workers who had been exposed to the radiation. The first case of cancer following chronic ulceration from exposure to roentgen rays was recorded only seven years after the discovery of this energy (Frieben). Acute constitutional symptoms were first reported by Walsh. The general constitutional effects, commonly known as radiation sickness, are beyond the scope of this review.

It may be well, before considering the detailed radiation effects, to define certain terms. "Radiosensitivity" and "radioresistance" have been rather loosely used, chiefly in relation to tumors. In an article to be published elsewhere I have suggested that radiosensitivity be defined as a response (regression of tumor or gross degeneration of tissue) to an amount of ordinary therapeutic radiation of less than 2,500 r given in divided doses; that radioresponsiveness be considered as a response in the range from 2,500 r to 5,000 r, and that those tissues or tumors showing gross change only in the range above 5,000 r be considered as radioresistant.

II. THE EFFECTS OF RADIATION ON THE CELL

All too frequently it is assumed that death of the cell, or inhibition of mitosis, is the only effect of radiation. However, although cessation of mitosis is one of the most sensitive indicators, other evidences of sub-lethal doses of radiation are known, such as the cessation of phagocytic activity.

While it has been assumed by Bergonié and Tribondeau that well differentiated cells are resistant to radiation and undifferentiated cells are less resistant, this holds true only in a broad sense. Thus, the cortical neuron is a highly differentiated structure and is highly resistant to radiation. On the other hand, the polymorphonuclear leukocyte is well differentiated and yet fairly sensitive. Cells of some relatively undifferentiated malignant tumors may be extremely resistant.

The resistance of some protozoa to radiation is startling. Koehring noted no change in the giant ameba, Chaos chaos, with 4,225 millicurie hours of radon. Organisms which are sensitive in one stage may be very resistant in another stage (as Drosophila, the ova of which have a median lethal dose of 155 r, while the late pupae have a median lethal dose of 95,000 r) (Scott).

Variation in temperature may enhance or diminish radiation effects. Increase in temperature may increase radiosensitivity in Drosophila eggs (Packard) but has no effect on Calliphora eggs (Scott). Evans

2. Drury. Gilchrist. Leppin. Stevens.

found that the skin of the young rat was from five to ten times as resistant to roentgen ray injury at 0 C. as at 30 C. As yet but little work has been done with mammals in this field.

THE CYTOPLASM AND ITS CONSTITUENTS

Observations on the effect of radiation on cytoplasm in the absence of nuclear material are almost entirely lacking.[3] Henshaw showed that in Arbacia cells the slowing of cell division brought about by radiation is due entirely to nuclear changes. The pure cytoplasmic fragments, after separation by Harvey's method of centrifugation, showed no response to the amount of radiation affecting the nucleated fragments. Attention has been called by Failla (1937) and by Failla and Sugiura to the importance of vacuolation in the cytoplasm following irradiation. While their observations are based on the study of tumor cells, they probably hold for normal cells as well.

Mitochondria.—The mitochondria of normal animal cells are reported to be sensitive to radiation; this sensitivity varies with physiologic changes in the cell.[4] Hirsch, working on the pancreatic cells of the mouse and Gatenby and co-workers, working on sperm cells, believed that radiation shows mitochondria more sensitive than nuclei. This sensitivity may lead to their partial or total destruction (Wail and Frenkel).

Whitman, working with rat sarcoma 338, concluded that radium had no effect on mitochondria. Ludford, in an extensive series of studies on animals, transplantable mouse tumors and human tissue irradiated with nonlethal doses, indicated that mitochondria in nondegenerating cells were little affected but became more conspicuous. Fogg and Warren (1937) found that there was no significant change in the mitochondria in the nondegenerating cells of the rat tumor, Walker 256.

Golgi Apparatus.—There is only a small volume of literature on the effect of radiation on the Golgi apparatus. Ludford comprehensively covered the literature up to 1932. Working on a group of mouse tumors and on some human tissue, he found that with a nonlethal dose there occurred in some cases swelling and fragmentation of the Golgi apparatus. He associated this change in structure with the general hypertrophy of the cells caused by radiation, and that primarily of tumor cells.

Fogg and Warren (1937) found that radiation produced a roughly systematic series of structural changes in the Golgi apparatus in the period previous to the restoration of full mitotic activity of the non-

3. The very considerable literature on the effects of irradiation of mammalian red blood cells will be considered in chapter III.

4. Iasswoine. Nadson and Rochlin. Stenstrom.

degenerating cell. The configuration changed from a net to a mass, then to discrete particles, followed by restoration of a net.

Centrioles.—Very little attention has been paid to the effect of radiation on centrioles (Fogg and Warren, 1940). No observations have been reported on the effect of radiation on the centrioles of normal cells, although Maximow mentioned "crowded centrioles" in irradiated fibroblasts in inflammatory tissue.

THE NUCLEUS

Far more attention has been paid to the nucleus and its constituents than to any other of the cellular components. It was shown by Hertwig and his school that the nucleus with its chromatin is much more affected by radiation than is the cytoplasm. Not only will radiation cause degenerative changes, but it may also produce giant nuclei. These have been carefully studied in fibroblasts by Maximow and appear either to be incapable of mitosis or to divide abnormally. Maximow's studies were made on the basis of inflamed tissue, and he stated that neither the inflammatory stimulus nor the radiant energy alone produces the changes observed with the dosage used, but only a combination of the two. He warned of the inhibitory effect of radiation on inflamed connective tissue.

While nuclear calcification has been reported as occurring after irradiation of tumors, published observations of such change in normal tissues is lacking.

Just as the nucleus appears more sensitive than the rest of the cell, so nuclei in mitosis are more sensitive than nuclei in the resting stage (Gaskell; Holthusen). The inhibition of mitosis appears early. Mottram exposed Ascaris eggs to radium and found that eggs in mitosis were killed by roughly one eighth of the dose required to kill cells in the resting stage. He gives numerous excellent drawings illustrating broken chromosomes, lagging of chromosomes and asymmetry of mitosis referable to the radiation effect. Perthes in 1904 noted abnormal mitosis due to radiation effect. This was further demonstrated by P. Hertwig. Dustin showed that with roentgen radiation mitosis of fibroblasts and of tissue cultures was arrested within twelve minutes. Alterations in mitosis, he pointed out, were not specific for radiation but could be produced by other injurious substances. Kemp and Juul studied the effect of roentgen rays, radium, heat and ether on mitosis in tissue cultures. Their conclusions were similar to those of Dustin. Clumping of chromosomes, fragmentation of them and lagging were among the chief effects.

Politzer (1925) emphasized pyknosis and pseudoamitosis as initial effects. In his monograph on the pathology of mitosis, Politzer (1934) differentiated three phases of radiation effect: a primary stage with an immediate drop in the number of mitoses, with pyknosis and pseudoamitosis; an interval, depending on duration and dosage, during which

the tissue is relatively free from mitotic activity, and then a secondary effect recognizable through fragmentation or variation of chromosomes and formation of abnormal or multiple nuclei when mitotic activity has been resumed.

By ingenious isolation of nuclei, Marshak was able to show that radioactive phosphorus is taken up by the nuclei to a greater degree than by the cytoplasm in mouse lymphoma and in regenerating rat liver.

The early prophase, or the premitotic phase, is generally considered (Canti and Donaldson; Colwell) to be the phase of mitosis most sensitive to injury either by radium or roentgen rays (Strangeways and Hopwood), although there is some variation as different cells are used (Love). It is important to remember that germ cells are more sensitive than most somatic cells and that minute doses may have greater cumulative effect on the former. This will be further discussed in the chapter on radiation effects on the gonads. Not all mitoses are inhibited, even in a heavily irradiated tissue culture (Warren, 1937). The frequent appearance of multipolar mitosis in irradiated tissue is an evidence of radiation effect that deserves further study, especially as this type of mitosis usually leads to cell death.

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(To Be Continued)

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PATHOLOGIC EFFECTS PRODUCED BY DEFICIENCY OF SINGLE METALLIC AND NONMETALLIC ELEMENTS

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Pathologic changes in various tissues which can be produced by dietary deficiencies of one or more essential nutritive factors are steadily assuming greater importance. Much more attention, however, has been paid to the effects of restricting the intake of vitamins than to those produced by feeding diets deficient in single inorganic elements. It is the purpose of this paper to review published studies of the latter type of deficiency. For virtually all those essential elements that have been given detailed attention, interesting pathologic changes have been described. With respect to the few which have been shown to be essential merely because of their effect on growth, histologic studies have yet to be made, while with respect to some others more detailed investigations are much needed. However, the information which has already been gathered should indicate the value of further studies in this fascinating field.

At the present time there are seventeen elements which are considered to be essential for the animal organism: hydrogen, carbon, oxygen, nitrogen, sulfur, calcium, phosphorus, magnesium, sodium, potassium, chlorine, iron, copper, zinc, manganese, cobalt and iodine. These elements are not the only ones which are present under normal circumstances. For instance, in spectrographic studies of the tissues of newborn rats, Rusoff and Gaddum [1] found aluminum, barium, strontium and tin in all the animals and, in addition, lead and silver in about half. Sheldon and Ramage [2] in a similar study found rubidium to be a constituent of normal voluntary and heart muscle. Whether some of the last-mentioned elements will be placed on the indispensable list at a later date awaits further investigation.

For completeness it is of interest to point out that a number of other elements have been shown to be essential for the nutrition of certain plants. These are boron, silicon, molybdenum, scandium and gallium.

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2. Sheldon, J. H., and Ramage, H.: Biochem. J. **25**:1608, 1931.

Attempts to classify all these essential elements in order to bring out correlations between their atomic structure and their biologic essentiality have been made recently by Kollath [3] and Steinberg.[4] The latter has suggested a chemical periodic table based on shell and subshell of transition, atomic number and rank of the elements. If one examines the position of the 17 essential elements in the classic periodic system, it will be seen that for the most part they are found in the lower periods, indicating that they have low atomic weights. In contradistinction it will be seen that the metallic poisons, such as gold, mercury, lead and uranium, are in the latter periods and have much larger atomic weights. The most important elements, which comprise most foodstuffs and vitamins—carbon, hydrogen, oxygen and nitrogen—all are found in the first two periods of elements. Single deficiencies of any of these four elements would naturally lead to death in short order, and they will not be considered further in the discussion to follow.

SULFUR

The metabolism of sulfur is intimately bound up with that of the sulfur-containing amino acids, methionine and cystine. The former is an indispensable factor for normal growth of rats. In addition, it plays a role in biologic methylation, has lipotropic activity and is a precursor of cystine. The latter amino acid is a unit of the molecules of several hormones (insulin, pituitary principles), protein enzymes and certain chemical regulators (glutathione). It is a precursor of taurine for the synthesis of taurocholic acid of bile, and it participates in the detoxication of many aromatic compounds.[5] Although sulfur compounds have been the subject of a large number of reported biochemical studies, of which those relating to choline metabolism are especially important, few investigations have been directed to the tissue changes of sulfur-deficient animals.

However, those studies which have been made are extremely interesting and point to the need for further ones. The relation of a diet deficient in cystine to hair growth was investigated by Smuts and co-workers.[6] It was found that on such a diet rats showed inhibition of hair growth, and some of the hairs on microscopic examination revealed abnormal structure in that the medullary cells were broader and more loosely packed. The proportion of cortex (which contains

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4. Steinberg, R. A.: J. Agric. Research **57**:851, 1938.

5. Lewis, H. B., in Harvey Lectures, 1940-1941, Baltimore, Williams & Wilkins Company, 1941, p. 159.

6. Smuts, D. B.; Mitchell, H. H., and Hamilton, T. S.: J. Biol. Chem. **95**: 283, 1932.

sulfur) to medulla was less than normal. Histologic studies of the skin were not made. The question was further investigated by Heard and Lewis,[7] who found that the addition of methionine to a diet deficient in sulfur-containing amino acids resulted in slightly increased production of hair. In an interesting study Martin and Gardner[8] showed that cysteine stimulated growth of a hairy coat in a strain of rats with hereditary hypotrichosis.

Feeding rats a cystine-deficient diet, Weichselbaum[9] described a syndrome which developed six weeks after the animals had been placed on the deficient ration. The rats refused food and began to have marked curvature of the spine. Jaundice and cyanosis of the feet, ears and nose appeared. Before death partial paralysis of the throat occurred. Postmortem examination showed hemorrhages throughout the liver. Unfortunately, microscopic studies were not made.

The effect of a diet deficient in cystine and methionine on the growth of spontaneous mammary carcinoma has been studied by Voegtlin and associates.[10] Not only were the tumors inhibited, but the mice also failed to grow. Following administration of either cystine or glutathione, a striking stimulation of the growth of both the tumors and the mice was observed. Negative results were obtained by Taffel and Harvey[11] when they studied the effects of sulfur amino acid deficiency on the healing of stomach wounds in rats. The whole question of the significance of sulfhydryl compounds as a factor in growth has been reviewed by Hueper.[12]

A relation of cystine deficiency to hyperplasia of the forestomach of rats has been suggested by the work of Sharpless.[13] Lesions were produced by a diet low in protein and could be prevented by feeding cystine.

Summary.—It has been found that sulfur amino acid deficiency leads to changes in the hair. Other specific effects on the tissues have yet to be more fully elucidated.

CALCIUM

Despite the great number of studies of calcium metabolism, the first satisfactory investigation of uncomplicated calcium deficiency (with adequate vitamin D) was reported only recently. Boelter and Greenburg[14]

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10. Voegtlin, C.; Johnson, J. M., and Thompson, J. W.: Pub. Health Rep. **51**: 1689, 1936.
11. Taffel, M., and Harvey, S. C.: Proc. Soc. Exper. Biol. & Med. **41**:72, 1939.
12. Hueper, W. C.: Arch. Path. **17**:218, 1934.
13. Sharpless, G. R.: Ann. Surg. **106**:562, 1937.
14. Boelter, M. D. D., and Greenburg, D. M.: J. Nutrition **21**:61 and 75, 1941.

found that when rats were placed on a diet containing about 0.01 per cent calcium the growth of these animals after four to five weeks was retarded. After seven to ten weeks the animals showed decreased sensitivity and reactivity. However, though the serum calcium fell to about 5 mg. per hundred cubic centimeters, tetany never appeared. Paralysis of the hindlegs was noted, and when the animals were stimulated by galvanic shocks, collapse and paralysis occurred. Sixty per cent were dead within twenty-three weeks. At autopsy there were widespread hemorrhages; the extravasation of blood was prominent in the nervous systems of those showing paralysis. The bones were extremely rarefied, and the percentage of ash and the calcium content were about half the normal. It is interesting to note that when calcium salts were injected into control and deficient animals, they were innocuous to the former while rupture of the right ventricle was seen in the latter rats. Detailed histologic studies of the tissues of such calcium-deficient animals are greatly to be desired.

PHOSPHORUS

Despite the numerous studies of the skeletal system involved in high calcium–low phosphorus rickets there have been few observations on the effects of diets adequate in all known factors (especially vitamin D) except phosphorus. A detailed histologic study was reported by Follis, Day and McCollum [15] of rats whose diets were adequate in calcium and vitamin D but contained only 0.017 per cent phosphorus. Apart from an extreme retardation of growth the only specific change that could be found was the early appearance of typical rickets. The thorax became greatly deformed and its capacity diminished. In consequence the lungs were atelectatic, and respiratory difficulty ensued. In the other tissues no changes were found except those interpreted as due to inanition. Metabolic studies on such animals by Day and McCollum [16] showed that a significant amount of phosphorus was mobilized from the bones and redeposited in the soft tissues, although there was a continuous negative phosphorus balance. In another study, Schneider and Steenbock [17] noted renal calculi after their rats had been on a low phosphorus diet (0.04 per cent) for seven weeks. Recently Freeman and McLean [18] described the effects of a phosphorus-deficient diet in dogs. Severe rickets was observed.

Summary.—The sole effect which has been observed in uncomplicated phosphorus deficiency is on the skeletal system. Severe rickets develops in both rats and dogs, even in the presence of adequate vitamin D.

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15. Follis, R. H., Jr.; Day, H. G., and McCollum, E. V.: J. Nutrition **20**:181, 1940.

16. Day, H. G., and McCollum, E. V.: J. Biol. Chem. **130**:269, 1939.

17. Schneider, H., and Steenbock, H.: J. Urol. **43**:339, 1940.

18. Freeman, S., and McLean, F. C.: Arch. Path. **32**:387, 1941.

SODIUM

The manifestations of sodium deprivation on rats were reported by Orent-Keiles, Robinson and McCollum.[19] Growth was retarded; death occurred in twenty weeks. Lesions were found in the eyes, and there were disturbances in reproductive function. Subsequent more detailed studies of the tissues of animals placed on the diet low in sodium failed to reveal any specific changes other than those in the ocular apparatus.[20] The first alteration to be noted was dilatation of the ducts of the meibomian glands, and as time went on this increased with coincident atrophy of the acinar elements. The ducts were apparently blocked by a crust which formed on the lids. The epithelium lining the inner margin of the lid became transformed into a stratified squamous type with loss of the goblet cells normally present. In the cornea there was leukocytic infiltration in the substantia propria just beneath the epithelium. This became more extensive, and blood vessels then grew into the cornea. The corneal epithelium subsequently became keratinized. Turpeinen[21] studied the effects of sodium deficiency in dogs. In these animals there were loss of weight, dryness of the skin and loss of hair. They survived eight weeks on the diet. The eyes were normal.

Summary.—It has been shown that in rats on sodium-deficient diets changes occur in the ocular apparatus. The other tissues are normal.

POTASSIUM

Lesions resulting from potassium deficiency have been studied by a number of investigators. In 1937 Schrader, Prickett and Salmon[22] described extensive changes in rats on a low potassium regimen. The diet of these rats was not entirely adequate in other nutritive essentials. Ascites appeared in the deficient animals, and there was hydrothorax, as well as hydropericardium, in some. The heart showed tiny opaque areas grossly. Microscopic examination of these revealed destruction of muscle fibers and replacement by connective tissue. The kidneys were enlarged and pale. Microscopically, the tubular epithelial cells were vacuolated and edematous and their nuclei pyknotic. The glomeruli were normal. There was atony of the intestinal tract. The mesenteric lymph nodes and Peyer's patches were enlarged, and the intestinal villi were distended by mononuclear cells. Similar myocardial lesions were noted in rats by

19. Orent-Keiles, E.; Robinson, H. A., and McCollum, E. V.: Am. J. Physiol. **119**:651, 1937.

20. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: Arch. Path. **33**:504, 1942.

21. Turpeinen, O.: Am. J. Hyg. **28**:104, 1938.

22. Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: J. Nutrition **14**:85, 1937.

Thomas, Mylon and Winternitz,[23] and these investigators produced identical changes in pigs. However, they postulated that in addition to a deficiency of dietary potassium there must also be one of vitamin B_6 to produce the myocardial lesions. Cardiac and renal lesions in rats were briefly reported by Heppel.[24] Using mice, Liebow, McFarland and Tennant[25] observed necroses in the myocardium. These investigators also noted extensive renal changes, which consisted of an increase in weight associated with hypertrophy and proliferation of the epithelium of the tubules, a focal increase in interstitial connective tissue associated with necrosis of isolated epithelial cells, and dilatation of the tubules. The kidney regained its normal weight and appearance when potassium was added to the diet. Changes were noted also in the voluntary muscle.

It is of interest to note that Thomas and co-workers[23] and Sykes and Alfredson[26] have described electrocardiographic changes in pigs and calves, respectively, on diets of low potassium content.

Using a purified diet adequate in all known nutritive factors except potassium, Follis, Orent-Keiles and McCollum[27] reported histologic studies on the tissues of rats which had been on the deficient diet for varying times. Changes specifically due to potassium deficiency and not to inanition were described in the heart and the kidneys. After the rats had been on the diet for eight days, it was found that occasional myocardial fibers lost their striations and became necrotic. Leukocytes then appeared, and the site was replaced by connective tissue. By the end of the second week the change was prominent. There were large areas of necrosis, sometimes covering two low power microscopic fields; these were noted principally in the ventricular musculature. It was interesting to find that even at the end of the observation period (three hundred and twenty-nine days), fresh lesions could be seen side by side with healed ones. When the skeletal muscles were examined, no changes were noted. This was all the more interesting since chemical analyses[28] of the heart and voluntary muscle showed a lower potassium content in the latter tissue. In the kidneys of the animals the first change was found after eight days of the deficient diet. Small vacuoles, which stained with scarlet red, appeared in the cytoplasm of the cells of the convoluted

23. Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Yale J. Biol. & Med. **12**:345, 1940.

24. Heppel, L. A.: Am. J. Physiol. **127**:385, 1939.

25. Liebow, A. A.; McFarland, W. J., and Tennant, R.: Yale J. Biol. & Med. **13**:523, 1941.

26. Sykes, J. F., and Alfredson, B. V.: Proc. Soc. Exper. Biol. & Med. **43**:575, 1940.

27. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: Am. J. Path. **18**:29, 1942.

28. Orent-Keiles, E., and McCollum, E. V.: J. Biol. Chem. **140**:337, 1941.

tubules. The cells then became necrotic and were sloughed off into the lumen of the tubule. These structures subsequently became lined by flattened regenerated epithelium. By the sixth week one could find many dilated tubules lined by such epithelium, and in some there was a bluish-staining granular material. As time went on, the dilatation became greater, and the kidneys consequently increased in size. The individual cells were hypertrophied. Studies of the urine of these animals were not made.

In man, disturbances in potassium metabolism are seen in familial periodic paralysis and Addison's disease. In the former syndrome the serum potassium drops during attacks though there is no loss of this cation from the body. No histologic studies of the cardiac musculature have been reported, but electrocardiographic changes have been described by Smith.[29] When an excessive amount of desoxycorticosterone is administered in the treatment of Addison's disease, the serum potassium falls as the serum sodium rises. Cardiac failure has been described,[30] though it may be due to other factors.

Summary.—From the evidence at hand it seems clear that potassium deficiency leads to necrosis of the cardiac musculature and to damage of the renal tubular epithelium.

MAGNESIUM

The syndrome of magnesium deficiency in rats and dogs was first reported by McCollum and co-workers.[31] The initial effect of diets low in magnesium was marked dilatation of the cutaneous vessels, especially those of the ears and paws; this lasted about a week. As the hyperemia began to disappear, extreme hyperirritability appeared so that fatal tonic-clonic convulsions occurred, initiated by the slightest external stimulus. Subsequent studies by Greenburg and Tufts[32] showed that auditory stimuli were most effective in producing the seizures and that their seat was probably in the midbrain. McCollum and co-workers found that if the convulsive attacks were survived, edema and trophic changes of the skin developed. Chemical studies on the blood showed a reduction in serum magnesium and calcium with a marked increase in serum cholesterol esters. The tissues of magnesium-deficient animals showed an increase in calcium content. The findings described have been confirmed by other investigators, using diets varying somewhat in magnesium and calcium content.

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30. Ferrebee, J. W.; Ragan, C.; Atchley, D. W., and Loeb, R. F.: J. A. M. A. **113**:1725, 1939.

31. Kruse, H. D.; Orent, E., and McCollum, E. V.: J. Biol. Chem. **96**:519, 1932. Orent, E.; Kruse, H. D., and McCollum, E. V.: Am. J. Physiol. **101**: 454, 1932; J. Biol. Chem. **106**:573, 1934.

32. Greenburg, D. M., and Tufts, E. V.: Am. J. Physiol. **121**:416, 1938.

Several studies of various tissues from animals on magnesium-deficient diets have been reported. In the kidneys Cramer [33] noted extensive degeneration of the tubular and glomerular epithelium with an accumulation of calcareous material in the lumens of the tubules and glomerular spaces. Brookfield [34] reported studies on 3 rats fed a magnesium-deficient diet and 1 control. In the deficient group there were varying degrees of necrosis of the tubular and glomerular cells. Schrader, Prickett and Salmon,[22] whose diet was not adequate in all other respects, noted changes in the livers of one third of their animals. These had scarring and round cell infiltration in the periportal spaces, and in a few there were scattered areas of necrosis. The kidneys were normal. Watchorn and McCance [35] found deposition of calcium in the straight and collecting tubules of the kidneys of the rats they studied; this led to obliteration with cystic dilatation of the tubule above the level of the cast. There was virtually no interstitial inflammation, nor were glomerular changes noted. The skin, which was examined while the erythema was most intense, showed "focal areas of necrosis and ulceration of the epithelium with an acute inflammatory reaction in the subjacent dermis." The bones were reported normal. Greenburg, Lucia and Tufts [36] also studied the renal changes produced by magnesium deficiency in rats. In the tubular lumens there was hyaline material; there was some interstitial infiltration by mononuclear cells. In addition, the lumens of some of the tubules contained granular debris and a few calcified concretions. There were large concretions in distended tubules of the corticomedullary zone, and these structures were lined by atrophic epithelium. An increase in urinary volume and an increase in urinary protein with a coincident decrease in plasma protein were also noted.

The only other animal which has been examined histologically is the calf. Moore, Hallman and Sholl,[37] in a detailed study, noted extensive calcification throughout the tissues of this animal. The most prominent change was a deposition of calcium in the elastic tissue fibers of the mural endocardium, aorta and pulmonary arteries and in the internal elastic lamella of smaller arteries of the muscular type. In 2 of 11 calves there was calcification of the muscle fibers in the myocardium. There was calcification of the skeletal muscle bundles of the extremities in a few animals which were examined in this respect. Proliferation of connective tissue was found in the portal spaces of the liver. The kidneys showed

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34. Brookfield, R. W.: Brit. M. J. **1**:848, 1934.

35. Watchorn, E., and McCance, R. A.: Biochem. J. **31**:1379, 1937.

36. Greenburg, D. M.; Lucia, S. P., and Tufts, E. V.: Am. J. Physiol. **121**: 424, 1938.

37. Moore, L. A.; Hallman, E. T., and Sholl, L. B.: Arch. Path. **26**:820, 1938.

interstitial scarring and atrophy of the parenchyma; in one third of the cases there was extreme tubular necrosis with deposits of calcium in the lumens.

Studies of interest have been made on the teeth. Kline, Orent and McCollum [38] noted extreme hypertrophy of the gums of their rats; this was due to an increase in connective tissue fibers beneath the epithelium. Changes were noted in the ameloblastic layer, and there were striations in the dentin. Watchorn and McCance [35] also found that the dentin had a striated appearance due to irregularities in staining. The odontoblasts showed various degrees of degeneration and had disappeared in some areas. In the molars, pulp stones were present. Becks and Furuta [39] presented a detailed and well illustrated account of extreme regressive changes in the enamel epithelium, especially marked in the anterior teeth of their rats. A second paper [40] pointed out marked changes in the formation of enamel. The most satisfactory description of the dental alterations has been presented by Irving.[41] There was retardation in the calcification of the teeth, with stratification of the predentin and adventitious calcification of the enamel organ and of the odontoblasts.

A recent paper by MacCardle and co-workers [42] is of great interest since these investigators showed a lowered magnesium content both in the normal-appearing and in the affected portions of the skin of patients with chronic disseminated neurodermatitis. Further studies will be awaited with interest.

Summary.—It is evident that in rats with low intake of magnesium histologic alterations take place in the kidneys, skin and teeth. The reports on the renal changes are not entirely in agreement but it is fairly clear that damage of the tubular epithelium is produced. In the calf, widespread calcification has been noted. More detailed studies of all the tissues of rats and other animals on magnesium-deficient diets are certainly indicated.

CHLORINE

Orent-Keiles, Robinson and McCollum,[10] on reducing the dietary intake of chlorine in rats, which they observed for ninety days, noted a retardation of growth. They made no studies of the tissues. Marquis [43] has confirmed the effect on growth of a diet low in chlorine.

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40. Becks, H., and Furuta, W. J.: J. Am. Dent. A. **28**:1083, 1941.
41. Irving, J. T.: J. Physiol. **99**:8, 1940.
42. MacCardle, R. C.; Engman, M. F., Jr., and Engman, M. F.: Arch. Dermat. & Syph. **44**:429, 1941.
43. Marquis, M.: Compt. rend. Soc. de biol. **128**:449, 1938.

IRON

The importance of iron for the formation of hemoglobin has been known for a long time. Despite this, however, cellular changes occurring in the bone marrow and other tissues in animals on diets deficient in iron have not been studied in any detail. In the chronic hypochromic microcytic anemia of iron deficiency occurring in man, according to Wintrobe,[44] the bone marrow shows hyperplasia and reveals a relative as well as an absolute increase of normoblasts. The extent of this normoblastic hyperplasia is proportional to the degree of anemia.

COPPER

The importance of copper for the animal organism has been definitely shown by investigations at the University of Wisconsin. Hart and co-workers [45] proved that in the anemic rat copper is necessary for the restoration of hemoglobin to a normal level. Examinations of the tissues of experimental animals on purified copper-deficient diets have not been reported, however. Such studies might be especially illuminating since Schultze [46] demonstrated that in rats copper deficiency causes a decrease in the cytochrome oxidase of the liver, the heart and the bone marrow.

Evidence for a relation of copper deficiency to the graying of hair in rats has been brought forward by Keil and Nelson,[47] Gorter [48] and Free.[49] The last investigator demonstrated that graying caused by mineral deficiency was different from that produced by lack of an organic factor, which was thought to be pantothenic acid. Henderson and associates [50] have recently shown conclusively that this is the case.

One of the most interesting aspects of mineral deficiencies has come to light in studies on a disease of lambs called sway-back. This malady is seen in newborn and young animals; in some flocks the incidence varies from 10 to 90 per cent of the lambs born. The symptoms consist of spastic paralysis, especially of the hindlimbs, severe incoordination of movement and, in some instances, blindness. The course is usually fatal, and death results from an intercurrent infection. Studies in England by Dunlop and Wells [51] have shown that the incidence of this disease could be greatly reduced by feeding salt licks containing copper to ewes during gestation.

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44. Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1942.
45. Hart, E. B.; Steenbock, H.; Waddell, J., and Elvehjem, C. A.: J. Biol. Chem. **77**:797, 1928.
46. Schultze, M. O.: J. Biol. Chem. **129**:729, 1939; **138**:219, 1941.
47. Keil, H. L., and Nelson, V. E.: J. Biol. Chem. **93**:49, 1931.
48. Gorter, F. J.: Nature, London **136**:185, 1935.
49. Free, A. H.: Proc. Soc. Exper. Biol. & Med. **44**:371, 1940.
50. Henderson, L. M.; McIntire, J. M.; Waismann, H. A., and Elvehjem, C. A.: J. Nutrition **23**:47, 1942.
51. Dunlop, D., and Wells, H. E.: Vet. Rec. **50**:1175, 1938.

More important, however, from the pathologist's standpoint is the fact that several years before Innes [52] reported studies on the central nervous systems of 32 lambs affected with the disease. In the brains there was symmetric diffuse demyelination of the white matter. This varied from foci of microscopic size to the virtually complete destruction of myelin in the severest forms of sway-back. In the animals with the severe condition there were cavities which could be seen grossly. In the areas of myelin destruction the axis-cylinders had disappeared. There was a certain amount of gliosis in such regions. Destruction of myelin was not found in the midbrain, the cerebellum or the brain stem. Secondary degeneration of the motor tracts was present in the spinal cord. In lambs in which the disease was mild, the nerve cells appeared normal, while in the more severely affected lambs degenerative changes were found. No inflammatory reaction was seen save the occurrence of phagocytes filled with fat. Innes [52] called attention to the similarity of this disease to Schilder's disease in man. It is also of interest to note that Eggleston [53] found that the central nervous system is richer in copper than any other tissue except liver.

ZINC

The indispensability of zinc in the diet of the rat was first conclusively demonstrated by Todd, Elvehjem and Hart in 1934.[54] Besides a disturbance in growth, these investigators noted loss of hair about the neck and shoulders, extending sometimes to involve the entire ventral surface of the body.

Recently a detailed study of the tissues of rats placed on a diet extremely low in zinc was reported by Follis, Day and McCollum.[55] Growth was retarded. In the deficient animals living longest (eleven weeks) there was partial loss of hair over the dorsum of the body. These denuded areas were roughened and scaly. Microscopic examination revealed changes in several epithelial structures. The skin showed hyperkeratinization and thickening of the epidermis. Instead of being three or four cells in width, it was increased to eight or ten cells in thickness. There was intracellular and intercellular edema. In the denuded areas, the hair follicles were absent, and only a few mononuclear leukocytes were found to mark where they had been. However, the sebaceous glands persisted, though the individual cells composing these structures were larger than normal. This change was found also about the nose but not on the ears or the plantar surface. The histologic picture is

52. Innes, J. R. M.: Rep. Inst. Animal Path. Cambridge **4**:227, 1934.

53. Eggleton, W. G. E.: Biochem. J. **34**:991, 1940.

54. Todd, W. R.; Elvehjem, C. A., and Hart, E. B.: Am. J. Physiol. **107**:146, 1934.

55. Follis, R. H., Jr.; Day, H. G., and McCollum, E. V.: J. Nutrition **22**:223, 1941.

unique and does not resemble that of any of the other nutritional dermatoses which have been reproduced in the rat. MacCardle and co-workers [42] have shown that zinc is a constant constituent of skin. In the esophagus of the zinc-deficient animal there was likewise thickening of the epithelial lining cells. Instead of being several layers in width as in the normal rat, the structure was six or eight epithelial cells in thickness. Normally at the point of keratinization a sharp transition is seen. In the deficient animals there was a thick layer of partially keratinized cells with pyknotic nuclei lying in a homogeneous pink-staining material. This was interpreted as due either to retardation in normal keratinization of the epithelium or to increased proliferation of cells. The change is similar to that which is seen in human psoriasis. It is interesting to note that the rest of the alimentary tract was normal except for foci of slight change in the buccal cavity. Another change which was noted in 2 of the 7 deficient animals was that of vascularization and leukocytic infiltration of the cornea. This resembled the change described by Bessey and Wolbach [56] in riboflavin deficiency and by Follis, Orent-Keiles and McCollum [20] in sodium-deficient rats.

In connection with these experiments on zinc deficiency, it is of interest to add that studies have been reported on the relation of zinc to certain enzyme systems. Keilin and Mann [57] noted that the red blood cell enzyme, carbonic anhydrase, contains about 0.3 per cent zinc. However, both Hove, Elvehjem and Hart [58] and Day and McCollum [59] failed to find any decrease in the activity of this enzyme in their deficient animals. Another study of zinc-deficient rats by Wachtel and co-workers [60] revealed no decrease in the uricase activity of the liver but did indicate that there was an increase in the uric acid content of the blood of the animals.

Summary.—It has been shown that zinc is necessary for normal growth of certain epithelial structures (skin and esophagus). It is likely that more prolonged and more severe deficiencies might lead to additional changes.

MANGANESE

The effects produced by restricting the dietary intake of manganese were first described by Orent and McCollum.[61] Growth was normal in the rats which they studied. The females exhibited normal estrus

56. Bessey, O. A., and Wolbach, S. B.: J. Exper. Med. **69**:1, 1939.

57. Keilin, D., and Mann, T.: Nature, London **144**:442, 1939.

58. Hove, E. C.; Elvehjem, C. A., and Hart, E. B.: J. Biol. Chem. **136**:425, 1940.

59. Day, H. G., and McCollum, E. V.: Proc. Soc. Exper. Biol. & Med. **45**:282, 1940.

60. Wachtel, L. W.; Hove, E.; Elvehjem, C. A., and Hart, E. B.: J. Biol. Chem. **138**:361, 1941.

61. Orent, E., and McCollum, E. V.: J. Biol. Chem. **92**:651, 1931.

cycles and when mated with normal males gave birth to approximately the usual number of young. However, they appeared indifferent to their young and failed to suckle them. When normal newborn animals were given them to nurse, they failed likewise to suckle them. If the young from manganese-deficient mothers were placed with normal females, a few were raised; all were undersized and of inferior appearance. From this Orent and McCollum [61] concluded that manganese deficiency might lead to abnormal functioning of the mammary tissues. The males on the manganese-deficient diet showed atrophy of the testes. There was immobility of the spermatozoa after one hundred days on the diet, and sterility was demonstrated by mating these males with stock females.

Using a manganese-low diet of different composition, Daniels and Everson [62] came to somewhat different conclusions. Almost half of the young born to manganese-deficient mothers were born dead or died in a few hours after birth. Of 37 rats given stock animals to nurse, 25 died within a few days; 12 were raised but were of inferior appearance. However, in contrast to Orent and McCollum's [61] observations the manganese-deficient mothers were able to raise practically all the foster young that were given them. Thus it seemed that the cause of death was in the young and not in the mothers. Histologic studies of the young from manganese-deficient female rats have not been reported.

However, Barnes, Sperling and Maynard [63] have made roentgenograms of the bones of animals on diets low in manganese. These studies were initiated by reports of osseous changes in chicks, which are described in the following paragraph. Barnes and associates noted in 2 of 16 rats born to females reared on a diet low in manganese that the tibia was shorter than normal, the epiphysis at the proximal end was thin and the diameter of the shaft at this end was larger than normal. In general, however, the findings of these investigators were not very convincing, since the paired feeding method was not employed.

The role of manganese in the skeletal growth of chicks was first established by Wilgus, Norris and Heuser [64] when they demonstrated that this element prevented the development of a disease called perosis. The picture was further complicated when Hogan, Richardson and Patrick [65] showed that an organic factor was also necessary, and this factor was identified as choline by Jukes.[66] More recently, Richardson

62. Daniels, A. L., and Everson, G. J.: J. Nutrition **9**:191, 1935.

63. Barnes, L. L.; Sperling, G., and Maynard, L. A.: Proc. Soc. Exper. Biol. & Med. **46**:562, 1941.

64. Wilgus, H. S., Jr.; Norris, L. C., and Heuser, G. F.: J. Nutrition **14**:155, 1937.

65. Hogan, A. G.; Richardson, L. R., and Patrick, H.: J. Nutrition (supp.) **19**:12, 1940.

66. Jukes, T. H.: J. Nutrition **20**:445, 1940.

and Hogan [67] have postulated that a third factor, as yet unidentified, is also necessary to protect chicks against perosis.

The term "perosis" is used to designate a deformity of the leg bones which may occur in chickens, turkeys, pheasants, grouse and quail. Grossly, one finds enlargement of the tibial-metatarsal joint, twisting and bending of the distal end of the tibia and of the proximal end of the metatarsus and slipping of the gastrocnemius tendon from its condyles. Little else is known about the disease save that the percentage of bone ash is normal. The blood serum calcium and phosphorus are normal, and both grossly and roentgenologically the change does not resemble rickets.[68] The need for histologic studies of perotic bones produced by deficiencies of the three factors mentioned is obvious.

Biochemical studies have produced some interesting results on the relation of manganese to perosis. Wiese and associates [69] showed in manganese-deficient chicks that the serum and bone phosphatase values were lowered. More recently, Combs, Norris and Heuser [70] found that if chicks are placed on rachitic diets and manganese is also omitted in some, the bone phosphatase decreases to approximately normal levels in the manganese-deficient group, while it remains elevated in the chicks on the uncomplicated rachitic diet.

Summary.—The offspring of manganese-deficient mother rats are either born dead or are too weak to suckle. Males on a manganese-deficient diet show atrophy of the seminiferous tubules. No other changes have been studied in rats. In chicks manganese is one of three factors the lack of which leads to a disease of the skeleton called perosis.

COBALT

A number of years ago Waltner and Waltner [71] reported that when cobalt is administered to rats, polycythemia is produced. This phenomenon has been confirmed in other animals as well. It seemed possible, therefore, that changes might be produced in laboratory animals by restricting the dietary cobalt. In 1938 Underwood and Elvehjem,[72] using a diet containing only 6 micrograms of cobalt per kilogram, concluded that at this level no effects had been produced. Further studies on dogs

67. Richardson, L. R., and Hogan, A. G.: Proc. Soc. Exper. Biol. & Med. **48**: 459, 1941.

68. Titus, H. W.: Poultry Sc. **11**:117, 1932.

69. Wiese, A. C.; Johnson, B. C.; Elvehjem, C. A.; Hart, E. B., and Halpin, J. G.: J. Biol. Chem. **127**:411, 1939.

70. Combs, G. F.; Norris, L. C., and Heuser, G. F.: J. Nutrition **23**:131, 1942.

71. Waltner, K., and Waltner, K.: Klin. Wchnschr. **8**:313, 1929.

72. Underwood, E. J., and Elvehjem, C. A.: J. Biol. Chem. **124**:419, 1938.

by the University of Wisconsin workers [73] showed, however, that cobalt did stimulate hemopoiesis in some anemic animals. This is the only laboratory evidence to date of the indispensability of cobalt in nutrition.

However, investigators of farm animals have shown that this element is necessary for the formation of blood in sheep and cattle. A disease called "enzootic marasmus" or "coast disease" develops when the soil content of cobalt is low.[74] The malady is characterized by emaciation, changes in the coat and anemia. Excessive amounts of iron are found in the liver, spleen and kidneys,[75] and this element disappears from these tissues when cobalt is administered. Unfortunately, there are no very satisfactory studies of the tissues of such cobalt-deficient animals. Neal and Ahmann [76] reported their findings in several calves suffering from the disease in Florida. The muscles were pale, and there was a diminution in body fat. The myocardium showed "degenerative" changes. The spleen was shrunken, and the pulp was decreased in amount. There was atrophy and fatty infiltration of the central liver cells. Obviously, detailed descriptions of all the tissues of more animals are urgently needed.

Summary.—Cobalt has been shown to have a specific relation to the formation of blood. In farm animals, a definite syndrome has been described, and this has been shown to be cured by the administration of cobalt.

IODINE

The relation of iodine to the thyroid gland was first shown by Baumann,[77] when he demonstrated this element in thyroid tissue. Since then there has accumulated a tremendous literature dealing with the effect of iodine on the thyroid.

The production of goiters in newborn puppies following removal of almost all their mothers' thyroid tissue early in pregnancy was reported by Halsted.[78] Marine and Lenhart [79] demonstrated that these congenital goiters could be prevented by administering iodine to the mothers during gestation. Marine and Kimball [80] later showed that changes in the thyroids of normal puppies could be produced by giving or withholding traces of iodine in the diet. Smith [81] reported that the administration

73. Frost, D. V.; Elvehjem, C. A., and Hart, E. B.: J. Nutrition **21**:93, 1941.
74. Underwood, E. J., and Filmer, J. F.: Australian Vet. J. **11**:84, 1935.
75. Underwood, E. J.: Australian Vet. J. **10**:87, 1934.
76. Neal, W. M., and Ahmann, C. F.: J. Dairy Sc. **20**:741, 1937.
77. Baumann, E.: Ztschr. f. physiol. Chem. **21**:319, 1896.
78. Halsted, W. S.: Johns Hopkins Hosp. Rep. **1**:373, 1896.
79. Marine, D., and Lenhart, C. H.: Arch. Int. Med. **4**:253, 1909.
80. Marine, D., and Kimball, O. P.: J. Lab. & Clin. Med. **3**:40, 1917.
81. Smith, G. E.: J. Biol. Chem. **29**:215, 1917.

of potassium iodide to sows prevented the birth of hairless cretin-like pigs. The relation of iodine to endemic goiter in fish was shown by Marine and Lenhart,[82] and the results of iodine therapy on the incidence of goiter in man have been recently summarized by McClendon.[83] Rienhoff [84] described the changes which take place in the human hyperplastic gland following the administration of iodine.

From a review of the foregoing facts it seemed probable that by using purified diets of low iodine content the exact relation of this element to the histologic structure of the thyroid gland could be elucidated with ease. This, however, has not been the case, since most investigators have been interested only in the weight and the iodine content of the gland, and only few histologic studies have been made. Then, too, the structure of the gland may be influenced by so many other factors, such as environmental temperature,[85] type of foodstuff [86] and excessive dietary calcium,[87] that one must be cautious in accepting the results unless all possible sources of error have been controlled.

Coplan and Sampson,[88] using a purified diet, found that the epithelial cells in the thyroids of their iodine-deficient rats became more columnar and the colloid content of the follicles decreased. This was followed after about twelve weeks by a phase in which the follicles increased in size and the epithelial cells became flattened. Chapman,[89] whose illustrations are more convincing, used a somewhat less purified diet and found that in the iodine-deficient animals the thyroid cells were more columnar and the glands had increased in size. The cytoplasm of the cells became vacuolated, and at some points there was invagination into the lumens of the follicles. The vascularity of the gland increased, and the intrafollicular stroma was likewise more abundant. Chapman's period of observation was sixty-five days. The effects of iodine deficiency may be accentuated by increasing the calcium content of the diet. Detailed histologic studies have been reported by Hellwig,[87] by Thompson [90] and by Levine, Remington and Kolnitz.[91] These investigations are of interest because they indicate what might occur if the iodine concentration of a diet of normal calcium content could be further reduced.

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82. Marine, D., and Lenhart, C. H.: J. Exper. Med. **12**:311, 1910.

83. McClendon, J. F.: Iodine and the Incidence of Goiter, Minneapolis, University of Minnesota Press, 1939.

84. Rienhoff, W. F.: Bull. Johns Hopkins Hosp. **37**:285, 1925.

85. Starr, P., and Roskelley, R.: Am. J. Physiol. **130**:549, 1940.

86. Chesney, A. M.; Clawson, T. A., and Webster, B.: Bull. Johns Hopkins Hosp. **43**:261, 1928.

87. Hellwig, C. A.: Arch. Path. **11**:709, 1931.

88. Coplan, H. M., and Sampson, M. M.: J. Nutrition **9**:469, 1935.

89. Chapman, A.: Endocrinology **29**:680, 1941.

90. Thompson, J.: Arch. Path. **16**:211, 1933.

91. Levine, H.; Remington, R. E., and Kolnitz, H.: J. Nutrition **6**:325, 1933.

Summary.—It has been demonstrated that iodine-deficient diets lead to hyperplastic changes in rats' thyroid glands. Further more detailed and prolonged laboratory studies of other animals are certainly needed to clarify the role of iodine in endemic goiter.

ELEMENTS NECESSARY FOR PLANT GROWTH

A large literature has accumulated bearing on the effect of metallic and nonmetallic deficiencies on the growth of plants. In the following paragraphs, only a few examples will be cited. As may be probable with respect to animals, some plants need certain elements which others do not.

Boron.—Up to the present time boron has not been shown to be indispensable to the animal organism.[92] However, it is necessary for the growth of some plants and furnishes a beautiful example of some of the complexities of botanic problems. Brenchley and Thornton [93] demonstrated the importance of boron for the normal metabolism of the leguminous broad bean plant. Normally nitrogen-fixing bacteria attach themselves to the root and multiply. Their presence causes the cells of the root to proliferate rapidly and form a nodule. From the circulatory system of the plant vascular strands grow into the nodule in order to supply foodstuffs for the bacteria, while they in turn provide nitrogenous compounds which they have synthesized. In culture mediums containing no boron there is a failure of the vascular strands to grow into the nodule. Consequently there are fewer and smaller nodules. More important, however, the bacteria injure the plant. Since the organisms receive no nourishment, the plant fails to grow, as it has no way of getting the nitrogenous compounds which are ordinarily furnished by the bacteria.

Silicon.—Evidence for the indispensability of silicon has been brought forward by Raleigh [94] among others. It was shown that a variety of table beet will not develop unless silicon is present in the culture medium.

Molybdenum, Gallium and Scandium.—Steinberg has reported interesting studies on the influence of these three trace elements on the fungus Aspergillus niger. He has demonstrated the indispensability of molybdenum,[95] gallium [96] and scandium.[97] The last element exhibited

92. Hove, E.; Elvehjem, C. A., and Hart, E. B.: Am. J. Physiol. **127**:689, 1939. Orent-Keiles, E.: Proc. Soc. Exper. Biol. & Med. **44**:199, 1940.

93. Brenchley, W. E., and Thornton, H. G.: Proc. Roy. Soc., London, s.B **98**: 373, 1925.

94. Raleigh, G. J.: Plant Physiol. **14**:823, 1939.

95. Steinberg, R. A.: J. Agric. Research **52**:439, 1936.

96. Steinberg, R. A.: J. Agric. Research **57**:569, 1938.

97. Steinberg, R. A.: J. Agric. Research **59**:749, 1939.

specificity when the organisms were grown on glycerol; growth was then doubled.

SUMMARY

From histologic studies of the tissues of animals placed on diets deficient in single inorganic elements valuable information has been gained on the functional relation of some of the elements to certain structures. For instance, the importance of potassium for the integrity of the myocardial fibers and of the renal tubular epithelium, of sulfur for normal growth of hair, of zinc to epithelium and of copper to the nervous system have been shown fairly definitely. More marked changes have been noted in potassium and magnesium deficiency than in sodium deficiency. It will be recalled that the former two elements are mainly intracellular, while the latter is found in the extracellular fluids. The need for further studies, with diets deficient in one element or in combinations of the necessary elements, is clearly shown. The importance of employing purified diets and of ruling out the factor of inanition by paired feeding in studies of this nature must be especially stressed.

Johns Hopkins Hospital.

Notes and News

Appointments.—G. Burroughs Mider has been appointed assistant professor of pathology in Cornell University Medical College, New York.

Among the newly elected members of the National Academy of Sciences are C. A. Elvehjem, University of Wisconsin; Michael Heidelberger, Columbia University; E. E. Tyzzer, Harvard Medical School, and S. A. Waksman, Agricultural Experiment Station, New Brunswick, N. J.

David R. Morgan has been appointed associate professor of pathology in Jefferson Medical College of Philadelphia.

Deaths.—Marcus W. Lyon Jr., pathologist, South Bend, Ind., died May 19 of coronary disease, aged 67.

Bernhard Fischer-Wasels, professor of pathologic anatomy in the University of Frankfort on the Main, in Germany, and editor of the *Frankfurter Zeitschrift für Pathologie,* has died at the age of 65 years.

Society News.—The Federation of American Societies for Experimental Biology, composed of the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, the American Society for Experimental Pathology, the American Institute of Nutrition and the American Association of Immunologists, has begun (1942) the publication of the *Federation Proceedings.*

Four issues will be published annually. Each year the March issue will contain the complete federation program of the scientific sessions of all the component societies as prepared for the forthcoming annual meeting, with abstracts of all scientific papers to be presented; the June and September issues will contain the full text of twenty or more papers presented at the annual meeting, including probably the papers on the joint society program and papers of several society symposiums; the December issue will contain material pertinent to the federation membership, i. e., the names of the officers and the membership list, together with an index of the completed volume.

The subscription price is $4 ($4.75 foreign), payable in advance. Subscriptions should be sent to Dr. D. R. Hooker, Managing Editor, 19 West Chase Street, Baltimore, Md.

Awards and Grants.—The Francis P. Garvan Gold Medal, honoring women in chemistry, has been awarded by the American Chemical Society to Florence B. Seibert, associate professor of biochemistry at the Henry Phipps Institute of the University of Pennsylvania, for "distinguished work on the chemistry of tuberculosis."

The exhibit by Philip Levine, Peter Vogel and E. M. Katzin on isoimmunization, the Rh blood factor and erythroblastosis foetalis received the gold medal of the American Society of Clinical Pathology at its meeting in Philadelphia in June.

The Columbia Foundation of San Francisco has made a grant of $20,000 in aid of research in ophthalmology under the direction of Charles Weiss and F. H. Rodin. The project includes the study of the bacteriology and immunology of the eye.

Book Reviews

The Biology of the Negro. Julian Herman Lewis, Ph.D., M.D., associate professor of pathology, University of Chicago; member of the Otho S. A. Sprague Memorial Institute for Medical Research; senior attending pathologist, Provident Hospital, Chicago. Pp. 443, with 17 tables. Price $5. Chicago: University of Chicago Press, 1942.

This book undertakes to compare diseases in Negroes and white peoples. It is in fact the first systematic treatise on Negroes from the point of view of comparative racial pathology. The title of the book might well have been the "Comparative Pathology of the Negro." In it are reviewed the observations in periodicals, reports and textbooks on the differences in the diseases of the two races, with special attention to the influences of biologic and environmental differences.

The first chapter deals with the vital statistics of the Negro population in the United States. It gives a good summary of the origin and growth of this population, its trends, sex composition, racial mixtures and birth and death rates. Next are presented excellent reviews of the anatomic, biochemical and physiologic characteristics that are distinctive of the Negro race.

The longest chapter is that on medical diseases as they differ in their manifestations in the Negro and the white race. Here tuberculosis in African as well as in American Negroes receives thorough consideration. The much greater incidence and mortality of tuberculosis in Negroes are explained by some writers as due to an inheritable susceptibility on the part of the Negro, by others as dependent on external factors, such as living conditions and accessibility to infection. The able analysis of the facts leads the author to stress the circumstance that Negroes "have not been in contact with tuberculosis long enough to develop a racial immunity through the forces of natural selection. But any explanation must also take into account the hazards of the living conditions of the large part of the Negro population." Like tuberculosis, syphilis in the Negro is greatly influenced by social conditions and subject also to modifications as "a community resistance is developed." Certain peculiarities of syphilis in Negroes may be explained by the strains of the spirochetes causing the disease as well as by severe physical labor on the part of the victims. Also reviewed are the incidence and manifestations in the Negro of venereal lymphogranuloma, malaria, leprosy, acute infections, hookworm infestation, diabetes and cardiovascular, renal, mental, blood and deficiency diseases. The only disease that seems to occur in the Negro only is sickle cell anemia, and, it is pointed out, the reasons for such racial segregation are not known.

The main topics in the section on surgical diseases are: the Negro as a surgical risk, peptic ulcer, prostatic hyperplasia, goiter, tumors. The analysis of the growing literature on cancer in the Negro brings out well the indications of differences of cancer in white and Negro peoples, a subject that invites further investigation.

There are chapters on obstetrics and gynecology, dermatology, ophthalmology and otolaryngology and dentistry.

The copious references to the literature are printed at the bottom of the pages. Much unnecessary labor and space have been saved by omitting the titles of articles in periodicals. There are good subject and author indexes. The book illustrates craftsmanship of high order.

The work will be a landmark in its field. It presents clearly and competently what is known of important comparative aspects of disease in the Negro. Whether racial peculiarities of disease are due to inherent, inheritable factors or to environmental conditions may be difficult to determine in a given disease, e. g., syphilis or cancer. "One must determine first of all in each case if the disease behavior

cannot be accounted for by the kind of houses people live in or the kind of work they do or the food they eat." Comparative study of disease along these broad lines will yield results of direct practical as well as scientific value.

Necropsy: A Guide for Students of Anatomic Pathology. Bela Halpert, M.D., assistant professor of pathology and bacteriology, Louisiana State University School of Medicine, and visiting pathologist, Charity Hospital of Louisiana at New Orleans. Cloth. Pp. 75. Price, $1.50. St. Louis: The C. V. Mosby Company, 1941.

In the preface the author points out that the various technics have been reviewed recently by Farber in his book, "The Postmortem Examination" (Springfield, Ill., Charles C. Thomas, Publisher, 1937); also that the principles involved and the relative merits of the various methods have been summarized by Mallory in his "Pathological Technique" (Philadelphia, W. B. Saunders Company, 1938). He then states that in 1924 he published a method based on removal and examination of the organs according to Ghon's *Pathologisch-anatomische Sektionsmethode*. The text in this new book is also based on Ghon's method. The topography and the anatomy of the various organs are considered in detail with special attention to regional lymph nodes and tributary blood vessels. "The material is arranged in the sequence in which the necropsy is performed to facilitate the correlation of the observations with their recording. Finally, a method of assembling and evaluating the data is illustrated by sample necropsy records." Mainly the book will be of help to beginners in necropsy work.

Textbook of Histology. Alexander A. Maximow, late professor of anatomy, University of Chicago, and William Bloom, professor of anatomy, University of Chicago. Fourth edition. Pp. xv and 695 with 562 illustrations. Price $7. Philadelphia: W. B. Saunders Company, 1942.

Previous editions of this book have been reviewed in the Archives of Pathology (**11**:330, 1931; **27**:659, 1939), so that a detailed review of the fourth edition is not necessary. There has been extensive revision, and new illustrations have been added. The book has established itself as an excellent text for students and as an authentic one volume reference work for graduates. Since this is a journal of pathology, perhaps it is not out of order to express the opinion that this book prepares medical students for the study of pathology better in cytology than in histology. The remedy suggested is not to stress cytology less but to emphasize histology more. To one who must teach pleurisy and diseases of the intestines, as examples, low power illustrations of these structures would be valuable. The publisher is to be commended for binding a laboratory book in such a way that the opened pages lie flat, as well as for handsome work in other respects. This continues to be a text that can be highly recommended.

Immunochemistry. William C. Boyd, Enrique E. Ecker, Michael Heidelberger, Sanford B. Hooker, Forrest E. Kendall, Stuart Mudd, L. Pillemer, Joseph E. Smadel, Theodore Shedlovsky and Charles A. Zittle. Reprint from the Annals of the New York Academy of Medicine (**43**:33-122, 1942). Price $1.25. New York: New York Academy of Sciences, 1942.

This reprint contains the papers presented at the conference on immunochemistry held by the Section of Chemistry and Physics of the New York Academy of Sciences, March 28 and 29, 1941, under the chairmanship of Michael Heidelberger. The subjects of the papers and the authors are: Antigens of Vaccinia, Joseph E. Smadel and Theodore Shedlovsky; Purification and Properties of the Protein of the "M-Substance" of a Group of Hemolytic Streptococci, Charles A. Zittle and Stuart Mudd; Complement, Enrique E. Ecker and L. Pillemer; The Quantitative Relationship Between Antigen and Antibody in the Precipitin Reaction, Forrest E. Kendall; Equine Antihemocyanin, Sanford B. Hooker and William C. Boyd. As pointed out by the chairman, the variety and the scope of the papers illustrate the forward movement in the field of immunochemistry.

Books Received

THE BIOLOGY OF THE NEGRO. Julian Herman Lewis, Ph.D., M.D., associate professor of pathology, University of Chicago; member of the Otho S. A. Sprague Memorial Institute for Medical Research; senior attending pathologist, Provident Hospital, Chicago, Ill. Pp. 443 with 17 tables. Price $5. Chicago: University of Chicago Press, 1942.

NECROPSY: A GUIDE FOR STUDENTS OF ANATOMIC PATHOLOGY. Bela Halpert, M.D., assistant professor of pathology and bacteriology, Louisiana State University School of Medicine, and visiting pathologist, Charity Hospital of Louisiana at New Orleans. Pp. 75. Price $1.50. St. Louis: C. V. Mosby Company, 1941.

SPONTANEOUS AND EXPERIMENTAL LEUKAEMIA IN ANIMALS. Julius Engelbreth-Holm, M.D., director of the cancer research laboratory of the Danish Anti-Cancer League; chief pathologist of the Finsen Institute and Radium Station of Copenhagen. Pp. 245. Price 15 shillings. Edinburgh and London: Oliver & Boyd, 1942.

DIRECTORY OF MEDICAL SPECIALISTS CERTIFIED BY AMERICAN BOARDS, 1942. Pp. xvi + 2,495. Price $7. New York: Columbia University Press, 1942.

OCCUPATIONAL TUMORS AND ALLIED DISEASES. W. C. Hueper, M.D., assistant director and principal pathologist, Warner Institute for Therapeutic Research, New York. Pp. 896. Price $8. Springfield: Charles C. Thomas, publisher, 1942.

TEXTBOOK OF PATHOLOGY. Sir Robert Muir, M.D., Sc.D., LL.D., F.R.S., emeritus professor of pathology, University of Glasgow; honorary pathologist to the Western Infirmary, Glasgow. Fifth edition. Pp. 991 with 599 illustrations. Price $10. Baltimore: Williams & Wilkins Company, 1941.

CARCINOMA AND OTHER MALIGNANT LESIONS OF THE STOMACH. Waltman Walters, M.D., M.S. in Surgery, D.Sc., F.A.C.S., surgeon, Mayo Clinic; Howard K. Gray, M.D., M.S. in Surgery, F.A.C.S., surgeon, Mayo Clinic; James T. Priestley, M.D., M.S. in Experimental Surgery, Ph.D. in Surgery, F.A.C.S., surgeon, Mayo Clinic; and associates in the Mayo Clinic and Mayo Foundation, Rochester, Minn. Pp. 576 with 143 illustrations. Price $8.50. Philadelphia and London: W. B. Saunders Company, 1942.

A STUDY OF THE BLOOD IN CANCER WITH SPECIAL REFERENCE TO THE NEEDS OF THE TUMOUR CLINIC. O. Cameron Gruner, M.D. (Lond.), author of "Biology of the Blood Cells" and "Studies in Puncture-Fluids." Pp. 100 with 40 illustrations. Price $4. Montreal, Canada: Renouf Publishing Company, 1942.